Lambda 20/Lambda 40 Operation and Parameter Description

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Safety Information 1

Safety Information

This manual contains information and warnings that must be followed by the user to ensure safe operation and to maintain the instrument in a safe condition.

Possible hazards that could harm the user or result in damage to the instrument are clearly stated at appropriate places throughout this manual.

The following safety conventions are used throughout this manual:

WARNING

We use the term WARNING to inform you about situations that could result in personal injury to yourself or other persons.

Details about these circumstances are in a box like this one.

CAUTION

We use the term CAUTION to inform you about situations that could result in ***serious damage to the instrument*** *or other equipment.*

Details about these circumstances are in a box like this one.

Translations of the warning messages used in this manual are given in *Translations of Warnings* on page 231.

Before using the instrument it is essential to read the manual carefully and to pay particular attention to any advice concerning potential hazards that may arise from the use of the instrument. The advice is intended to supplement, not supercede the normal safety code of behavior prevailing in the user's country.

IEC 1010 Compliance

This instrument has been designed and tested in accordance with IEC 1010-1: *Safety requirements for electrical equipment for measurement, control, and laboratory use,* and Amendment 1 to this standard.

CSA Compliance

This instrument meets the Canadian Standards Association (CSA) Standard CAN/CSA-C22.2 No. 1010.1-92: *Laboratory Equipment*.

UL Compliance

This instrument meets the Underwriter Laboratories (UL)
Standard UL 3101-1/Oct.93: *Electrical Equipment for laboratory use, part 1: general requirements.*

Electrical Protection

Insulation: Class I as defined in IEC 1010-1.

Installation Category: The instruments are able to withstand transient overvoltage according to Installation Category II as defined in IEC 1010-1 and IEC 664.

Pollution Degree: The equipment will operate safely in environments that contain non-conductive foreign matter and condensation up to Pollution Degree 2 as defined in IEC 1010-1 and IEC 664.

Electrical Safety

To ensure satisfactory and safe operation of the instrument, it is essential that the green/yellow lead of the line power cord is connected to true electrical earth (ground).

If any part of the instrument is not installed by a PerkinElmer service representative, make sure that the line power plug is wired correctly:

Terminal	Cord Lead Colors	
	International	**USA**
Live	Brown	Black
Neutral	Blue	White
Protective Conductor (earth/ground)	Green/Yellow	Green

WARNING

Electrical Hazard

Any interruption of the protective conductor inside or outside the instrument or disconnection of the protective conductor (earth/ground) terminal is likely to make the instrument dangerous.
Intentional interruption is prohibited.

Lethal voltages are present in the instrument

- *Even with the power switch OFF, line power voltages can still be present within the instrument.*
- *When the instrument is connected to line power, terminals may be live, and opening covers or removing parts (except those to which access can be gained without the use of a tool) is likely to expose live parts.*
- *Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

When working with the instrument:

- Connect the instrument to a correctly installed line power outlet that has a protective conductor (earth/ground).
- Do not attempt to make internal adjustments or replacements except as directed in this manual.
- Do not operate the instrument with any covers or parts removed.

- Servicing should be carried out only by a PerkinElmer service representative or similarly authorized and trained person.

- Disconnect the instrument from all voltage sources before opening it for any adjustment, replacement, maintenance, or repair. If, afterwards, the opened instrument must be operated for further adjustment, maintenance, or repair, this must only be done by a skilled person who is aware of the hazard involved.

- Use only fuses with the required current rating and of the specified type for replacement.

  Do not use makeshift fuses or short-circuit the fuse holders.

- Whenever it is likely that the instrument is no longer electrically safe for use, make the instrument inoperative and secure it against any unauthorized or unintentional operation.

The instrument is likely to be electrically unsafe when it:

- Shows visible damage.
- Fails to perform the intended measurement.
- Has been subjected to prolonged storage under unfavorable conditions.
- Has been subjected to severe transport stresses.

Electromagnetic Compatibility (EMC)

European Union (EMC Directives)

This instrument has been designed and tested to meet the requirements of the EC Directives 89/336/EEC and 92/31/EEC. It complies with the generic EMC standards EN 50 081-1 (rf emissions) and EN 50 082-1 (immunity) for domestic, commercial, and light industrial environments.

This instrument has passed the following EMC tests:

Emission: EN 50 081-1:92	Immunity: EN 50 082-1:92
Emission of conducted and radiated noise	*Electromagnetic Compatibility*
EN55 011:91 EN 60 555-2:87 EN 60 555-3:87	IEC 801-2:91 IEC 801-3:84 IEC 801-4:88 IEC 801-5:90

United States (FCC)

This instrument is classified as a digital device used exclusively as industrial, commercial, or medical equipment. It is exempt from the technical standards specified in Part 15 of the FCC Rules and Regulations, based on Section 15.103[c].

Japan (FCC)

This instrument has been tested and found to comply with the limits of a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. Changes or modifications not expressly approved by the manufacturer could void the user's authority to operate the equipment.

Environment

Operating Conditions

WARNING

Explosive Atmosphere

This instrument is not designed for operation in an explosive atmosphere.

The instrument will operate correctly under the following conditions:

- Indoors.
- Ambient temperature +15 ºC to +35 ºC.
- Ambient relative humidity 20% to 80%, without condensation.
- Altitude in the range 0 m to 2000 m.

Storage Conditions

You can store the instrument safely under the following conditions:

- Ambient temperature -20 ºC to +60 ºC.
- Ambient relative humidity 20% to 80%, without condensation.
- Altitude in the range 0 m to 2000 m.

When you remove the instrument from storage, before putting it into operation allow it to stand for at least a day under the approved operating conditions.

Chemicals

Use, store, and dispose of chemicals that you require for your analyses in accordance with the manufacturer's recommendations and local safety regulations.

WARNING

Hazardous Chemicals

Some chemicals used with this instrument may be hazardous or may become hazardous after completion of an analysis.

The responsible body (for example, Laboratory Manager) must take the necessary precautions to ensure that the surrounding workplace and instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA, ACGIH, or COSHH documents.

Venting for fumes and disposal of waste must be in accordance with all national, state and local health and safety regulations and laws.

OSHA: Occupational Safety and Health Administration (U.S.A.)
ACGIH: American Conference of Governmental Industrial Hygienists (U.S.A.)
COSHH: Control of Substances Hazardous to Health (U.K.)

Toxic Fumes

If you are working with volatile solvents or toxic substances, you must provide an efficient laboratory ventilation system to remove vapors that may be produced when you are performing analyses.

Waste Disposal

Waste containers may contain corrosive or organic solutions and small amounts of the substances that were analyzed. If these materials are toxic, you may have to treat the collected effluent as hazardous waste. Refer to your local safety regulations for proper disposal procedures.

Deuterium lamps and other spectral lamps are maintained under reduced pressure. When you dispose of lamps that are defective or otherwise unusable, handle them correctly to minimize the implosion risk.

UV Radiation

You should be aware of the health hazards presented by ultraviolet radiation.

- When the deuterium (UV) lamp is illuminated, do not open the spectrophotometer covers unless specifically instructed to do so in the manual.
- Always wear UV-absorbing eye protection when the deuterium lamp is exposed.
- Never gaze into the deuterium lamp.

Symbols Used on the Instrument

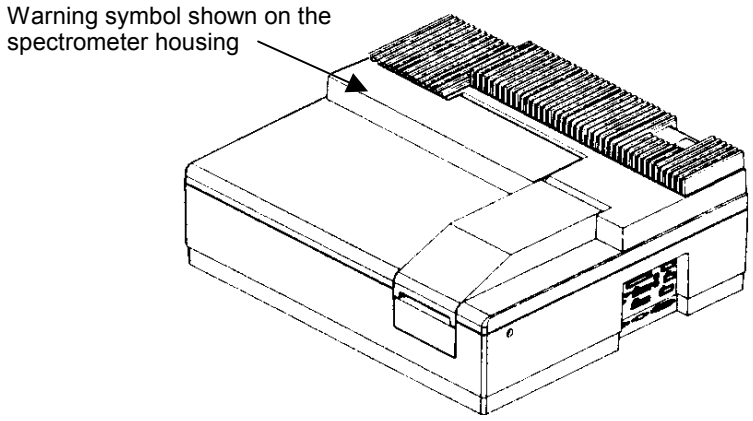


Figure 1 Lambda 20/40 Spectrometers

UV Radiation–Risk of Eye Damage
The lamp emits intense UV radiation which can damage your eyes.
Do not gaze into a lighted lamp.
Always wear UV absorbing safety glasses when looking at the radiation from the lamp.

Hot Surfaces–Risk of Burns
Lamps soon become hot and also heat up other components in the lamp compartment.
Allow the components to cool to room temperature before installing new lamps, or making adjustments in the lamp compartment.

Warning Labels on the Instrument

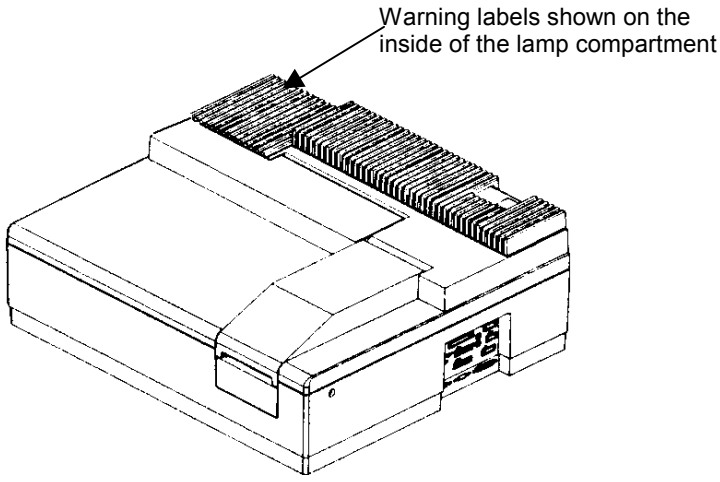


Figure 2 Lambda 20/40 Spectrometers

The following warnings are shown on the inside of the lamp compartment.

DANGER
HIGH
VOLTAGE

DANGER
HAUTE
TENSION

WARNING
UV RADIATION-HARMFUL TO THE EYES
HOT COMPONENTS – RISK OF BURNS

ACHTUNG
UV-STRAHLUNG-GEFÄHRDUNG DER AUGEN
HEISSE BAUTEILE –
VERBRENNUNGSGEFAHR

ATTENTION
RADIATION UV-DOMMAGEABLE POUR
LES YEUX – PARTIES CHAUDES
RISQUE DE BRULURES

Introduction 2

Introduction

The Lambda 20 and Lambda 40 are versatile spectrometers operating in the ultraviolet (UV) and visible (Vis) spectral ranges. The spectrometers have some common features.

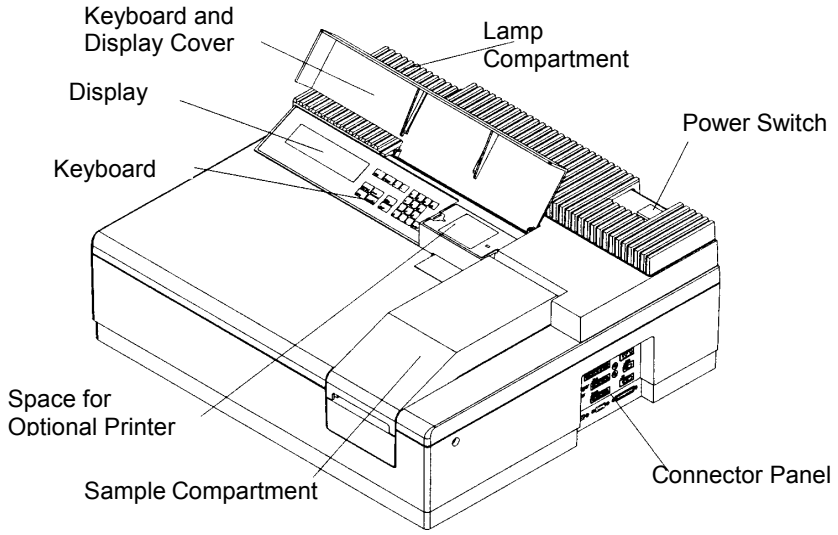
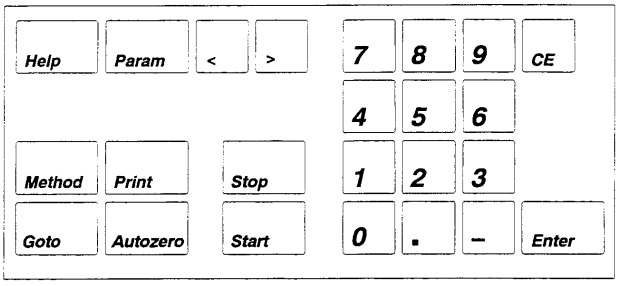


Figure 3 Features common to Lambda 20 and 40 Spectrometers

Keys



Key	Description
[HELP]	Provides additional parameter information on the display.
[PARAM]	Selects next parameter. Switches to next lower level.
[<] [>]	Selects previous or next element in a particular level.
[METHOD]	Selects methods. Use with numerical keys (see *Selecting a Method* on page 48).
[PRINT]	Prints out the top line of the current standby or method header display.
[STOP]	Stops a method. Switches to next higher level.
[GOTO]	To change the wavelength setting (see *Setting the Wavelength Manually* on page 41).
[AUTOZERO]	Starts autozero (background correction) (see *Manual Autozero* on page 42).
[START]	To start and continue a method.
[0] to [9]	Numerical keys.
[·]	Decimal point.
[-]	Minus, used to enter negative values.
[ENTER]	Confirms parameter.
[CE]	Clears unconfirmed parameter entry.

Key Combinations

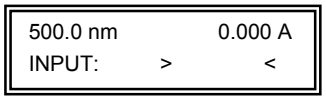
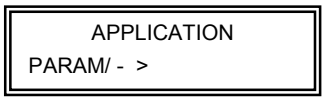
Key Combination	Description
[nnn] [METHOD]	Selects method nnn.
[nnn.n] [GOTO]	To change to wavelength nnn.n.
[nn] [PARAM]	Selects parameter nn when you are in the parameter list level.
[·] [PARAM]	Selects parameter tagging. Selects default methods from **Application** level.
[·] [PRINT]	Prints out a method or branch directory (see *Printing Out a Directory* on page 67).
[-] [PARAM]	Selects previous parameter.
[1] [PRINT]	Prints out method parameters.
[2] [PRINT]	Prints out a directory of the methods available in the branch.
[3] [PRINT]	Prints out the additional method information.
[4] [PRINT]	Line feed.
[5] [PRINT]	Form feed.
[6] [PRINT]	Prints out the Peltier cell holder temperature shown on the display.*
[7] [PRINT]	Prints out the spectrometer status.†
[7] [9] [·]	Full reset (see *Reset* on page 43).

* Functions only when the Peltier accessory is installed.

† Caution: All methods are deleted!

[1] [5] [-]	Switches to SuperUser mode (see *Activating SuperUser Mode* on page 207).

Displays

Display	Description
 Standby display	Standby display with wavelength and measured value. Starting point, appears after switch-on following initialization routine. → set up absorbance manually, set wavelength manually, select method, print out ordinate reading, print out method directory of the relevant branch, return to branch header
	Branch header with branch name. → select the branch method, change to another branch, select default method of branch, view help message, print out the spectrometer display, return to standby display.
	Method header with method number and type. → start method, select method processing, select another method, view help message, print out method parameters, print out help message, return to standby display.

Display	Description
MODIFY METHOD PARAM/ ->	Method processing with selected processing function. → start processing function, select another processing function, return to method header.
ORDINATE MODE A <-->	Parameter directory with parameter names and value. → change parameter, select next/previous parameter, start method, view help message for current parameter, return to method header.
WAVe.MAX 900.0 nm ENTER > <	Parameter with parameter names value and value entry field. → change parameter value, select next/previous parameter, start method, view help messages for current parameter, return to method header.

Displays shown during the measurement:

Display	Description
AUTOZERO START,STOP	Request to start autozero.
AUTOZERO XXX.X nm 0.000 A	Display during autozero.

Display	Description
NNNN SMPL n START,STOP,AUTOZERO	Request to start sample measurement. **NNNN** is the method name.
NNNN SMPL n START,STOP,AZ,PRINT	Request to start sample measurement, or replot graphics. Appears in methods with replot function when graphics plot set to yes. **NNNN** is the method name.
2 SCAN XXX.X nm 0.000 A	Display during sample measurement (**SCAN** method).
ORDINATE MODE &A <-->	Display of a tagged parameter. If necessary, the parameter can be changed.
AUTOZERO IN CELL1 START, STOP	Cell Changer: request to insert blank solutions and start autozero.
SAMPLES INTO 9-CELL START,STOP,AUTOZERO	Cell Changer; request to insert sample solutions and start the measurement.
AUTOZERO SIPPER ACCESSORY START	Sipper: request to press start key on the Sipper (autozero).
SAMPLE 1 SIPPER ACCESSORY START	Sipper: request to press start key on the Sipper (sample measurement).
REF 1 [XXX] START,STOP,AUTOZERO	Request to measure a reference solution.

3 Startup and Shutdown

Startup and Shutdown

When operating the spectrometer, wait until the **BUSY** display has disappeared before pressing the next key. This allows the software to complete the calculations and the motors to move the optics to their required setting.

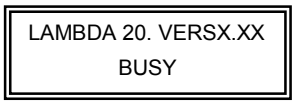
Before starting analysis, leave the spectrometer switched on for approximately 10 minutes to allow the lamps to warm up and stabilize.

Startup

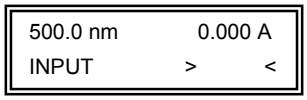
1. Open the sample compartment cover.
2. Make sure that the beam paths are free, that is,
   - No objects (for example, cables) project into the beam paths.
   - No samples are in the sample compartment.
   - Accessories are properly installed.

NOTE: *If the sample compartment is obstructed during the startup procedure, the spectrometer will not initialize correctly.*

3. Close the sample compartment cover.
4. Switch on the power switch.
5. Wait for the standby display to appear.
   Lambda 40 shows on those spectrometers.
   The standby display.
   Other values may be shown.
6. Switch on the accessories.



Initialization display



Standby display

Shutdown

1. Return the spectrometer to standby, use [STOP] or [PARAM].
2. Switch off the accessories.
3. Open the sample compartment cover.
4. Remove samples and cells from the sample compartment.
5. If accessories (for example, flowcell) are installed in the sample compartment clean them thoroughly.
6. Close the sample compartment cover.
7. Switch off the spectrometer.
   See also *Wakeup* on page 123.

Single Cell Holder

Description

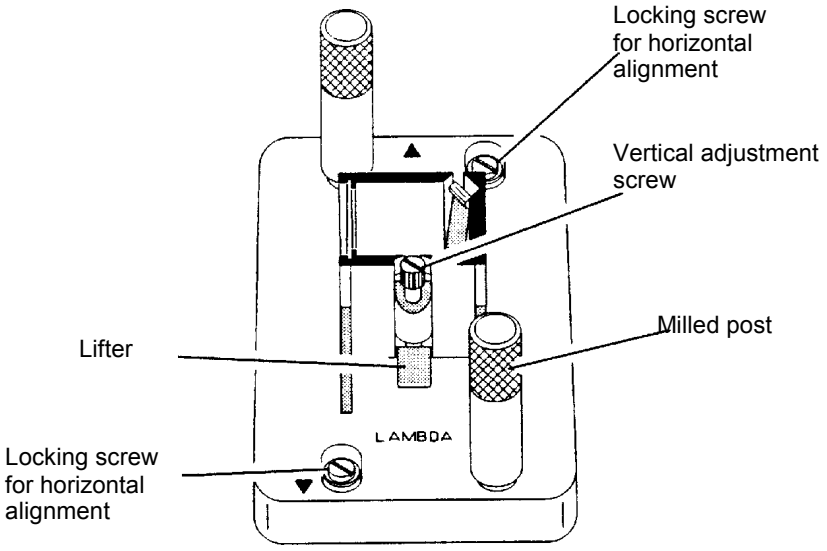


Figure 4 Single Cell Holder B0505071

NOTE: *Depending on the spectrometer, the single cell holder can be installed in two different positions in the sample compartment. Always install the holder such that the arrow on the cell holder lines up with the center point on the baseplate (see* Installing the Single Cell Holder *on page 32).*

Inscription legible on Holder	Use in Spectrometer
LAMBDA	In this position, the cell holder can be used with all Lambda Series Spectrometers.
BIO LAMBDA 2	In this position, the cell holder can be used with Lambda 2 Series Spectrometers as Lambda 10, 20, 40, Bio, (baseplate with 4 threaded holes). The smallest beam diameter is exactly in the middle of the cell. This is useful especially for operation with micro and semi-micro cells.

Installing the Single Cell Holder

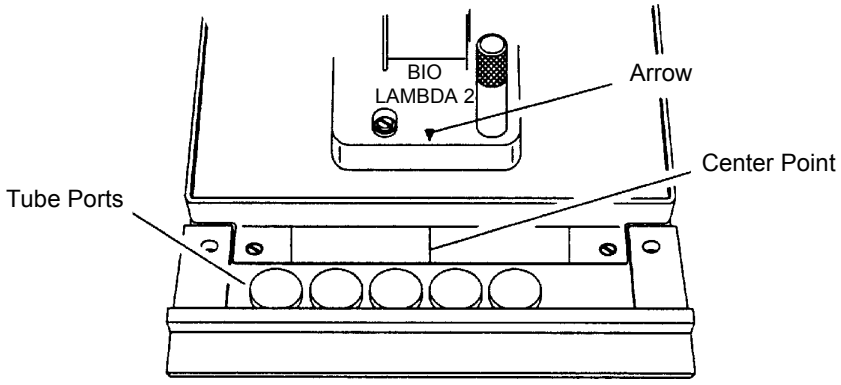
There are two single cell holders provided with the instrument, one for the sample beam and one for the reference beam.

Install the single cell holder in the sample compartment as follows:

1. Orientate the holder so that the lifter is toward the rear of the sample compartment.

2. Lower the holder so that the two alignment holes slip onto the two studs on the baseplate at the bottom of the sample compartment.

   The arrow on the cell holder must line up with the centre point of the baseplate, and **BIO LAMBDA 2** must be legible.



3. Move the milled posts a little to locate the threaded holes in the baseplate, and then tighten the milled posts.

   The tube ports located at the front of the sample compartment allow you to lead tubes from, for example, flowcells, water-thermsotatted cell holders, in and out of the sample compartment.

   When not in use, you should always insert the caps into the ports.

Aligning the Single Cell Holder

Coarse alignment of the single cell holder is carried out as follows:

1. Open the sample compartment cover.
2. Fill matching cells with a low-absorbing solvent (deionized water or ethanol).
3. Insert one cell into the sample cell holder and one into the reference cell holder. Make certain that the cell is pushed down fully.

NOTE: *The alignment procedure is for a given cell in a given holder. After alignment, the cell should always be used in the same holder.*

4. Block the sample and reference beam window on the right hand side of the sample compartment with a card to prevent white light from saturating the detector.

5. Return to standby display.

6. Using the [GOTO] key, slew the monochromator to 0 nm to obtain a beam of visible (zero order) radiation in the sample compartment.

7. By holding a piece of matt white paper behind each cell holder, visually examine the light spot to see that the radiation beam is passing through the cell sample area.

   Diffraction patterns become apparent if the radiation beam impinges on the cell wall.

8. If the radiation beam is not centered exactly, loosen the two locking screws and the two milled posts on the relevant cell holder and shift the cell holder plate to center the radiation beam horizontally.

   Then retighten the two milled posts and the two locking screws.

9. Now visually check the vertical alignment of the radiation beam in the cell sample area.

   Alignment is correct when the radiation beam is just above the floor of the cell sample area (min. 2 mm) or covers the cell window.

NOTE: *The center of the window for micro flowcells should be ideally approximately 15 mm above the base of the cell.*

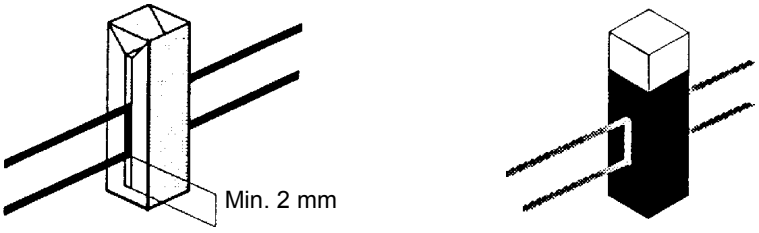


Figure 5 Correct Alignment of the Radiation Beam in the Cell Sample Area

10. If vertical alignment is required, turn the vertical adjustment screw on the lifter either clockwise to raise the cell, or counterclockwise to lower the cell.

11. Recheck the horizontal alignment of the radiation beam through the cell and correct if necessary.

12. Using the [GOTO] key, slew the monochromator to any value above 200 nm.

13. Remove the card blocking the sample beam window and close the sample compartment cover.

This completes the coarse alignment of the cell holder.

Fine Alignment

If fine alignment is necessary, proceed as follows:

1. Using the [GOTO] key, slew the monochromator to your measurement wavelength or to 460 nm.

2. Call up a method that uses transmission (%T) as the ordinate.
   If necessary change the ordinate mode to transmission.

3. Open the sample compartment cover.

4. Insert the cell with a low absorbing solvent into the sample cell holder.
   Leave the reference cell holder empty.

5. Make horizontal fine alignment to the sample cell holder (locking screws and milled posts loosened) to obtain the highest possible transmittance reading on the display.
   Close sample compartment cover while measuring transmittance.

6. Make vertical fine adjustment using the vertical adjustment screw again to obtain the highest possible reading.
   Close sample compartment cover while measuring transmittance.

7. When you are satisfied with the alignment, tighten the milled posts and the locking screws on the cell holder.

8. Insert the matching cell with the same low absorbing solvent into the reference cell holder.

   The first cell remains in the sample cell holder.

9. Repeat steps 5 through 7 with the reference cell holder, but this time obtain the lowest possible transmittance reading on the display.

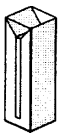
This completes the fine alignment procedure.

When the cell holder has been aligned once, you can take it out and reinstall it without aligning it again.

Minimum Volume Applications

To measure minimum sample volumes, use microcells (offered by PerkinElmer).

The minimum sample volume required is a function of the cell internal width or volume and is specified below.

Cell Type		Cell Internal Width	Pathlength	Minimum Volume Required	Part Number
	Height of liquid slightly more than height of beam	2 mm	1 cm	200 µL	B0631071 (pair)
		4 mm	1 cm	400 µL	B0631064 (pair)
		Cell Volume	**Pathlength**	**Minimum Volume Required**	**Part Number**
	Cell window completely filled with liquid	0.5 µL	0.01 cm	2 µL	B0631082
		2.5 µL	0.5 cm	5 µL	B0631080
		5 µL	0.1 cm	10 µL	B0631083
		5 µL	1.0 cm	10 µL	B0631081
		30 µL	1.0 cm	50 µL	B0631079

NOTE: *You should align microcells very carefully in the radiation beam by following the procedures in* Aligning the Single Cell Holder *on page 33. When aligning microcells, fill each cell with the minimum volume of liquid specified in the above table to make sure that the liquid meniscus is not in the radiation beam.*

4 Operating without Methods

Operating without Methods

Overview

Measurements are usually carried out using methods containing all the necessary parameters, see *Using Methods* on page 71.The following functions can be carried out via the keyboard:

- Setting the wavelength
- Manual autozero
- Quick sample measurement
- Reset
- Print
- Help

Setting the Wavelength Manually

The wavelength can be set manually in standby using the [GOTO] key as follows:

1. Set the spectrometer to the standby display.
2. Press [GOTO].
3. Enter the desired wavelength, for example, **325.5.**
4. Press [ENTER].
   The monochromator slews to the selected wavelength.

   OR

1. Set the spectrometer to the standby display.
2. Enter a wavelength using the numeric keys, for example, **325.5**.

3. Press [GOTO].
   The monochromator slews to the selected wavelength.

Manual Autozero

In this operation, the [AUTOZERO] key is used to set the measured absorbance value to 0, or transmittance value to 100%, for the actual wavelength shown on the display.

1. Open the sample compartment cover.
2. Place cells with blank solution in the reference and sample cell holders.

   OR

   Use air as blank.
3. Close the sample compartment cover.
4. Set the desired wavelength using [GOTO].
5. Press [AUTOZERO].

Autozero is complete once the standby display reappears.

The ordinate mode of the last used method always appears on the display. To convert absorbance to transmittance or vice versa, select a TIME DRIVE method, and then select the desired ordinate mode.

Quick Sample Measurement

You can make quick sample measurements as follows:

1. Prepare the sample.
2. Set the spectrometer to the standby display.
3. Press [GOTO].

4. Select your desired wavelength.
5. Press [ENTER].
   The monochromator slews to the selected wavelength.
6. Open the sample compartment cover.
7. Insert cells with blank solutions in the reference and sample cell holders.

   OR

   Use air as blank.
8. Close the sample compartment cover.
9. Press [AUTOZERO] and wait until the autozero is completed.
10. Open the sample compartment cover.
11. Remove the blank and insert the cell with sample solution in the sample cell holder.
12. Close the sample compartment cover.
13. The absorbance (A) or transmittance (%T) reading and wavelength are shown on the display.
    The ordinate mode of the last used method always appears on the display.
    Use a TIME DRIVE method to change from absorbance (A) to transmittance (%T).
14. Press [PRINT] to print out the reading.

Reset

By a full reset the spectrometer and its program are returned to the default condition. You can carry out a full reset at any time.

NOTE: *In carrying out a full reset, all methods will be erased. Before carrying out a full reset, make sure that all important methods are printed out.*

To carry out a full reset:

1. Switch off the spectrometer.
2. Press [7] [9] [•] (seven, nine, point) simultaneously.
3. Keep the keys pressed and switch on the spectrometer.
4. Keep the keys pressed until the display appears.
   The instrument requests the printer type.
5. Select the printer type and press [START].
   After the full reset is completed a status report is printed out (when a printer is connected).

NOTE: *There are default methods stored in the internal memory of the spectrometer. These methods are not deleted after a full reset and can be copied and amended (see* Selecting a Method *on page 48).*

Print

Press [PRINT] to print out the top line shown on the standby and method header displays.

Other functions using the print key are described in *Printing Out a Directory* on page 67.

Help

Press [HELP] to view additional information about the current method or parameter on the display.

Methods 5

What are Methods?

Methods are a collection of those parameters necessary for a particular analysis using the spectrometer and are stored as method files.

You can process large numbers of samples efficiently using the methods.

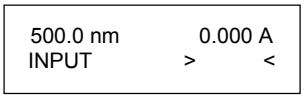
The parameter values necessary for the analysis in question need only be set once and are then available on request.

Up to 200 methods can be stored in the spectrometer; each method can be allocated a number between 1 and 999.

On delivery, 10 basic methods are programmed in the spectrometer; these are immediately available for use.

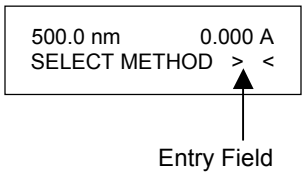
Selecting a Method

1. Switch to the standby display, using [STOP] or [PARAM].



Standby display

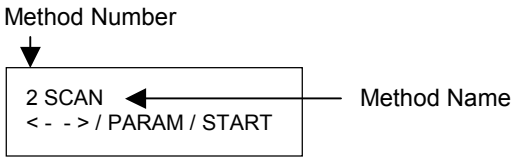
2. Press [METHOD].
3. Enter the method number.
4. Press [ENTER].



The method is loaded onto the operational memory.
The method header then appears on the display.

OR

1. In the standby display, enter the method number.
2. Press [METHOD].



Method Header

The method is loaded onto the operational memory.

The method header then appears on the display.

The method can now be used for measurement.

If an unused method number is entered, the function **NEW METHOD** appears.

A new method can now be created (see *Creating a New Method* on page 57).

If you don't know the method number, return to the standby display and use [PARAM] to switch to the first method header. Then use the arrow keys to view the available methods in turn.

Default Methods

Default methods are stored in the spectrometer. The default methods can be read and copied, but not modified.

The copied default methods can then be modified to suit your own requirements.

You access the default methods as follows:

1. Switch on the spectrometer in SuperUser mode (see *SuperUser* on page 207).
2. Press [STOP] repeatedly until the **APPLICATION** branch header is displayed.

   ```
   APPLICATION
   PARAM / < - - >
   ```

3. Press [•] [PARAM] to select the first of the default methods.
4. Use the arrow key to select the required method type.
5. Press [PARAM] and then use the arrow key to select **MARK FOR COPY**.
6. Press [STOP] to return to the **APPLICATION** branch.
7. Create an empty method file (see *Creating an Empty Method File* on page 57).

8. Copy the default method into the empty method file using the **NEW FROM MARK** parameter (see *Copying the Method into Another Method File* on page 64).

   The default method can now be amended as required.

Editing Methods

The following options are available:

- **MODIFY METHOD**
- **DELETE METHOD**
- **NEW METHOD**
- **NEW METHOD NAME**
- **CHECK METHOD**
- **MARK FOR COPY, NEW FROM MARK**
- **PRINT METHOD**

To recreate methods that have been inadvertently erased or written over, regularly print out all important methods.

Modifying a Method

1. Select the method to be modified.
2. Press [PARAM].
3. Press [PARAM] again.
4. Change the displayed parameter values if required.

   OR

   Press [PARAM] to select the next parameter.

   OR

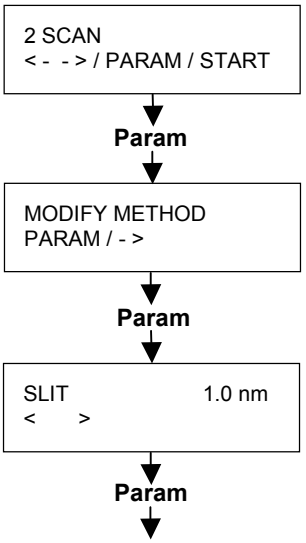
   Press [-] [PARAM] to recall the previous parameter.

   OR

   Enter the appropriate parameter number and press [PARAM] to select a particular parameter. See *Parameter Numbers and Descriptions* on page 169 for parameter description and parameter numbers.

   OR

   Press [STOP] to cancel.



Changing a Parameter

1. Select the parameter to be changed.
2. Depending on the parameter shown, change as described in the following table.

Text/Symbol	Procedure
< - - >	Select option with the arrow keys.
ENTER:	Enter the desired value with the numeric keys. Press [ENTER] to confirm the value entered.
- >ENTER:	Appears If several values have to be entered. Use the arrow key to select the desired parameter. Enter the required value. Press [ENTER] to confirm the value entered. For example: Several reference values have to be entered. Enter the value for **REF 1**. Confirm with [ENTER]. Use the arrow key to move to **REF 2**. Continue until values have been entered for all the references.

3. Press [PARAM] to proceed to the next parameter.

   You can press [HELP] for additional information about a parameter.

   When a particular parameter is set to **YES** the extra parameters required automatically appear in their correct order.

   For example, when **GRAPHICS PLOT** is **YES**, the extra parameters **ORD MAX**, **ORD MIN**, **SCALE** and **GRID** appear.

   When **GRAPHICS PLOT** is **NO**, the extra parameters **ORD MAX**, **ORD MIN**, **SCALE** and **GRID** no longer appear.

Tagging a Parameter

You tag a parameter to change it at appropriate times during the analysis, for example prior to the start of each sample measurement.

Untagged parameters can only be changed prior to the start of a method.

The following table shows the type of tagging, and when it appears during the analysis:

Tag	Symbol	Appears
BATCH	**!**	Prior to the start of each sample batch.
CALL	**&**	Prior to the start of a method.
START	*****	Prior to the start of each sample
FIX	**None**	No tag.

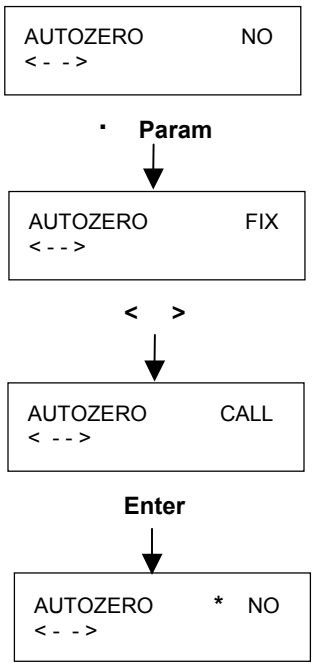
Tag a parameter as follows:

1. Select the parameter to be tagged.
2. Press [·] [PARAM].
3. Select the appropriate tagging with the arrow keys.

4. Press [ENTER] to confirm the tag.

   Every parameter can be tagged.

   For parameters where tagging is less meaningful (**LAMP, GRAPHICS PLOT**), tagging is accepted, but not carried out. The tagged parameter appears at the appropriate time, but cannot be changed.

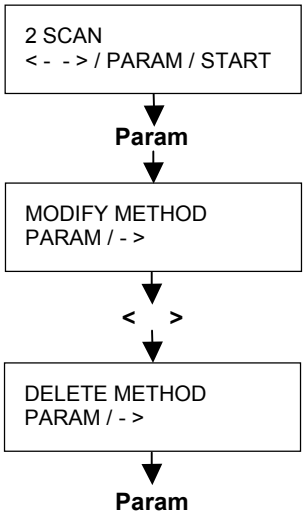


Deleting a Method

1. Select a method that can be deleted.
2. Press [PARAM].
3. Use the arrow keys to select **DELETE METHOD**.
4. Press [PARAM] again to delete the method.
   The method is deleted as soon as [PARAM] is pressed, and the display returns to the next method header in the list.

   OR

   Press [STOP] to cancel.



Creating a New Method

You can create a new method in one of the following ways:

- Create a new empty method file.
- Overwrite an existing method file.

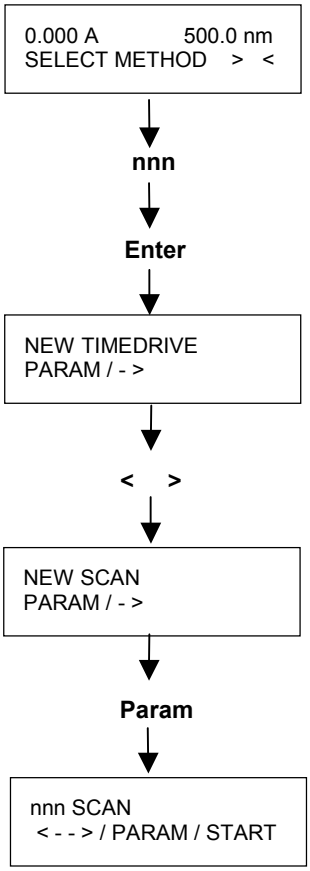
Creating an Empty Method File

1. Press [METHOD].
2. Enter a method number **nnn** not previously used.
3. Press [ENTER].
   The first method of the **NEW METHOD** level appears.
4. Use the arrow keys to select the required method type.
5. Press [PARAM].
   This confirms the creation of the new method.

   OR

   Press [STOP] to cancel.

6. Modify the new method as required.



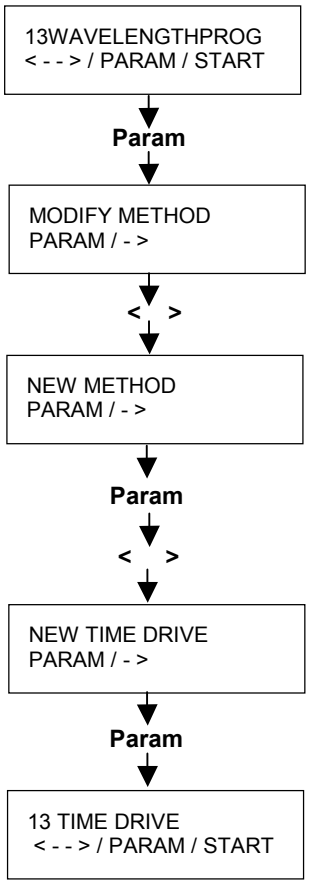
Overwriting an Existing Method

1. Select a method that can be overwritten.
2. Press [PARAM].
3. Use the arrow keys to select **NEW METHOD**.
4. Press [PARAM] again.

5. Use the arrow keys to select the method type.
6. Press [PARAM].
   The existing method is written over.

   OR

   Press [STOP] to cancel.
7. Modify the new method as required.



New Method Name

You can rename methods using the **NEW METHOD NAME** parameter.

The method number remains the same when you rename a method.

1. Select the method to be renamed.
2. Press [PARAM].
3. Use the arrow keys to select **NEW METHOD NAME**.
4. Press [PARAM] again.
5. Use the arrow keys to select letters.
   Confirm each letter by pressing [ENTER].

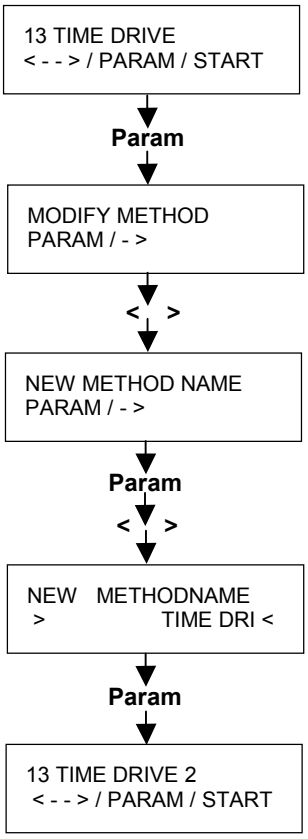
   OR

   Use the numeric keys to enter numbers.
   Confirm each number by pressing [ENTER].

   OR

   Press [ENTER] without entering a letter or number for an empty space.
   You can combine letters and numbers.

6. Press [PARAM] to confirm the new name.



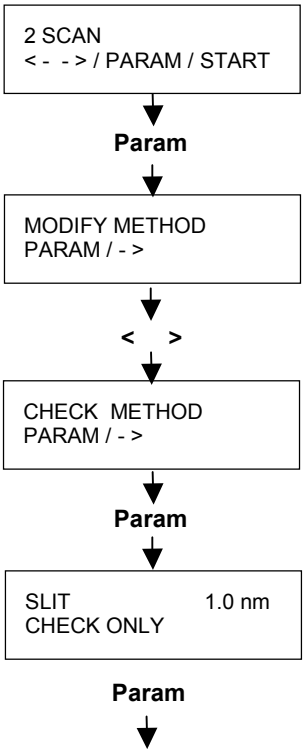
Checking a Method

When using the **CHECK METHOD** function, the parameter values are displayed, but cannot be changed.

1. Select the method to be checked.
2. Press [PARAM].
3. Use the arrow keys to select **CHECK METHOD**.
4. Press [PARAM] to check each parameter in turn.

   OR

   Press [STOP] to cancel.



Copying Method Parameters into a New Method File

This is useful when you wish to make a new method with only a few parameters different from the original.

Two steps are involved in this procedure:

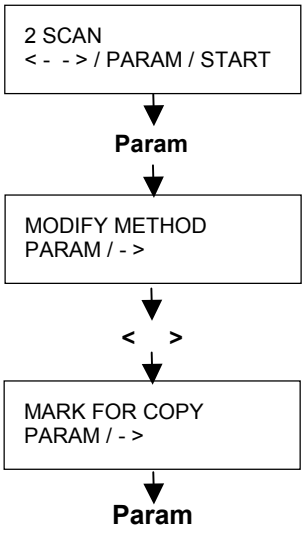
- Marking the method for copy.
- Copying the method into another method file.

Marking for Copy

1. Select the method whose parameters are to be copied.
2. Press [PARAM].
3. Use the arrow keys to select **MARK FOR COPY**.
4. Press [PARAM] to mark the method.
   The method is now marked for copying in the next step.

   OR

   Press [STOP] to cancel.



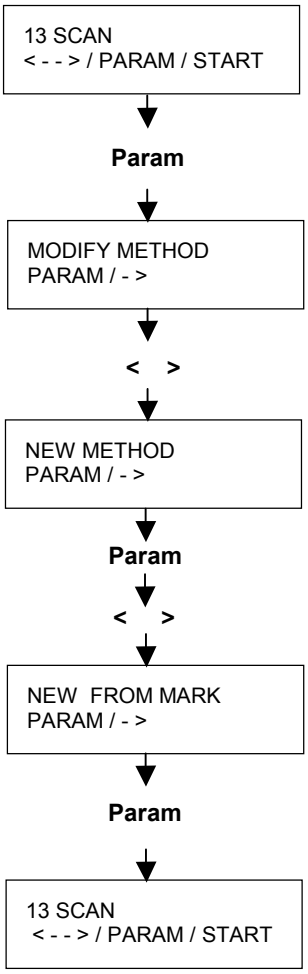
Copying the Method into Another Method File

1. Create a method (see *Creating a New Method* on page 57).
2. Press [PARAM].
3. Use the arrow keys to select **NEW METHOD**.
4. Press [PARAM] again.
5. Use the arrow keys to select **NEW FROM MARK.**
6. Press [PARAM].
   The parameters from the marked method are copied into the newly created method.

   OR

   Press [STOP] to cancel.

7. Modify the new method as required.



Printing Out a Method

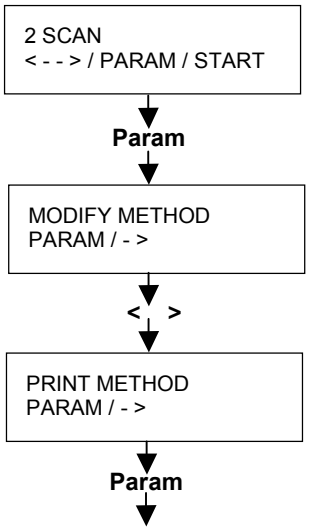
Printing out a method provides a list of its parameters and their current values.

A printer must be installed and configured (refer to the installation manual).

1. Select the method to be printed out.
2. Press [PARAM].
3. Use the arrow keys to select **PRINT METHOD**.
4. Press [PARAM] again to print out the method.

   OR

   Press [STOP] to cancel.
   You can also press [1] and then [PRINT] to print out the method parameters.



Printing Out a Directory

Printing out a directory provides a list of its methods.

A printer must be installed and configured (refer to the installation manual).

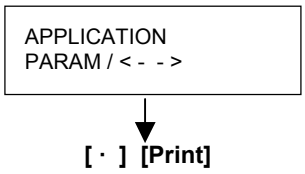
There are two directories, the spectrometer directory and the branch directory.

Spectrometer Directory

The spectrometer directory is a list of all methods for the spectrometer (including the SuperUser methods).

Print out the directory as follows:

1. Select a branch header.
2. Press [·] and then [PRINT] to print out a directory of the methods.



Branch Directory

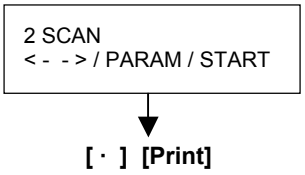
The branch directory is a list of all the methods in the selected branch.

Print out all the methods in the selected branch as follows:

NOTE: *To select SuperUser branches, you must first enter as SuperUser, see* SuperUser *on page 207.*

1. Select a method in the branch to be printed out.

2. Press [2] and then [PRINT].
   This prints out the branch directory.



Print Key

The Print key can be used on its own to print the top line of the standby or method header displays, or in combination with other keys to provide other functions, as listed in the following table.

Key	Description
[PRINT]	Prints out the current values shown on the top line of the standby and method header displays. Calls up the graphics plot parameters after each sample analysis (see *Replot* on page 79).
[1] [PRINT]	Prints out the method parameters.
[2] [PRINT]	Prints out a directory of methods available in the branch.
[3] [PRINT]	Prints out the additional method or parameter information.
[4] [PRINT]	Line feed.
[5] [PRINT]	Form feed.
[6] [PRINT]	Prints out the Peltier cell holder temperature shown on the display. Functions only when Peltier accessory is installed.
[7] [PRINT]	Prints out the spectrometer status.

Help Key

To view the additional information text for a particular method or parameter proceed as follows:

1. Select the desired method or parameter.
2. Press [HELP].
3. Continue to press [HELP] to view all the text.

   OR

   Press [STOP] to interrupt the help function.

NOTE: *Help text is available in German, refer to the print configuration in SuperUser.*

Using Methods 6

Methods Overview

The spectrometer incorporates the basic types of methods shown in the table below:

No.	Type of method	Use	Section
1	**TIME DRIVE**	Measurement over a certain period at one wavelength.	Single Wavelength Measurements page 82
2	**SCAN**	Scanning spectra and derivative spectra.	Scanning a Spectrum page 85
3	**WAVELENGTH PROG**	Measurement at several wavelengths; differential and ratio analysis at several wavelengths.	Measurement at Several Wavelengths page 88
4	**CONCENTRATION 1**	Determination of concentration using peak height.	CONCENTRATION 1 Method (Peak heights) page 91
5	**CONCENTRATION 2**	Determination of concentration using peak area or 2nd derivative.	CONCENTRATION 2 Method (Peak Areas, 2nd Derivative) page 96
6	**ENZYME KINETICS**	Enzyme kinetics.	Enzyme Kinetics page 105
7	**SUBSTRATE KIN**	Substrate kinetics.	Substrate Kinetics page 109.
-	**OLIGOQUANT 1**	Quantitative analysis of oligonucleotides up to 50 bases long.	Methods for Quantitative Analysis of Oligonucleotides page 115

No.	Type of method	Use	Section
-	**OLIGOQUANT 2**	Quantitative analysis of oligonucleotides longer than 50 bases.	Methods for Quantitative Analysis of Oligonucleotides page 115
900	**DATE/TIME**	To enter and change the date and time.	Date/Time page 121
901	**WAKEUP**	To switch on the lamps and allow them to warm up before the start of the working day.	Wakeup page 123
999	**SELF CHECK**	Instrument internal check of the topics.	Self Check page 125

Preprogrammed Methods

Using the preprogrammed methods you only need to change a few parameters to quickly create your own individual methods. A list of the preprogrammed methods is given in the table below:

No.	Type of Method	Use
800	**TIME DRIVE**	Measurement at 500 nm
801	**SCAN**	Absorbance scan from 900 nm to 200 nm.
802	**WAVPROG**	Wavelength program at 260 nm.
803	**FACTOR CONCENTR.**	Quantitative analysis without calibration. Absorbance at a specific wavelength is multiplied by a factor to give concentration of analyte.

No.	Type of Method	Use
804	**CALIB. CONC.**	Quantitative analysis measurement after calibration using 3 references.
805	**ONE REF. CONC.**	Quantitative analysis using 1 reference for calibration.
806	**TWO REF. CONC.**	Quantitative analysis using 2 references for calibration.
807	**THREE REF. CONC.**	Quantitative analysis using 3 references for calibration.
808	**CONC. VARIABLE**	Quantitative analysis measurement after calibration using 3 references.
809	**SURVEY SCAN**	Scans a survey spectrum (fast scan speed).
810	**ABSORBANCE SCAN**	Scans absorbance spectra.
811	**%-T-SCAN**	Scans transmittance spectra.
812	**UV-SCAN**	Scans absorbance spectra between 400 nm and 200 nm.
813	**VIS-SCAN**	Scans absorbance spectra between 900 nm and 400 nm.
814	**REPETITIVE SCAN**	Scans absorbance spectra between 400 nm and 200 nm.
815	**CYCLE WAVPROG**	Wavelength program at 260 nm.
816	**RATIO**	Ratio of absorbance readings at different wavelengths.

No.	Type of Method	Use
817	**DIFFERENCE**	Difference of absorbance reading at different wavelengths.

Method Procedure

When a method is selected, it can be used for measurements. When starting the method, the system automatically makes requests via the display.

Display	Request
AUTOZERO START,STOP	Autozero. Place a cell containing a blank solution in each of the sample and reference cell holders. OR Place an empty cell in each of the sample and reference cell holders (measurement against air). Press [START] to start the autozero.
SCAN SMPL n START,STOP,AUTOZERO	Sample measurement. Place the cell containing the sample solution in the sample cell holder. [**n**] [ENTER] can be used to switch directly to **SAMPLE n**. **n** is the sample number. Press [START] to start the measurement.
ORDINATE MODE & A < - - >	Tagged parameter (**&** is the **CALL** tag). If desired, enter a new value and press [ENTER]. OR Select a new value using the arrow keys. Press [START] to proceed with the analysis.

Analysis Procedure

1. Select the appropriate method (see *Selecting a Method* on page 48).
2. If necessary, modify the method parameters.
3. Press [START].
4. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

   OR
   If the display is:

   ```
   XXX                SAMPL n
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].
   XXXX is the method type.
5. Continue to insert samples when asked until they have all been measured.
   When the graphics plot parameter in the method is set to **YES**, the display is:

   ```
   XXX                SAMPL n
   START,STOP,AZ, PRINT
   ```

   This allows you to change the scale of the plot for the sample just analyzed, and replot the result (see *Replot* on page 79).

NOTE: *Pressing* [STOP] *interrupts a method in progress and returns you to the ready display***.**

Replot

You can replot sample data for last analyzed sample in certain methods when the graphics plot parameter in that method is set to **YES**.

Replot the data as follows:

1. Set the graphics plot parameter to **YES** in the method parameters before starting the method.

2. Start the method.
   The following ready display appears after the first sample analysis.

   ```
   XXXSCAN        SMPL 2
   START,STOP,AZ, PRINT
   ```

3. Press [PRINT].
   The following display appears:

   ```
   ORD MAX        0.000 A
   ENTER      >       <
   ```

4. Enter a new upper value for the ordinate, if required, and then press [ENTER] to confirm the value.

5. Press [START] to move to the next parameter.
   The following display appears:

   ```
   ORD MIN        0.000 A
   ENTER      >       <
   ```

6. Enter a new lower value for the ordinate, if required, and then press [ENTER] to confirm the value.

7. Press [START] to move to the next parameter.
   The following display appears:

   ```
   SCALE          50.0 nm/cm
   < - - >
   ```

8. Select a new abscissa scale value using the arrow keys, if required.

9. Press [START].

   ```
   REPLOT SPECTRUM
        BUSY
   ```

   The data is then replotted using the new ordinate and abscissa values.
   The display returns to the ready display.

   ```
   SCAN                SAMPL 2
   START,STOP,AZ, PRINT
   ```

   You can replot the data as often as required.
   Press [STOP] to interrupt the replot and return to the ready display.

NOTE: *Once you start the analysis for the next sample, the data for the previous sample is deleted from the spectrometer memory and replaced with that of the current sample being analyzed***.**

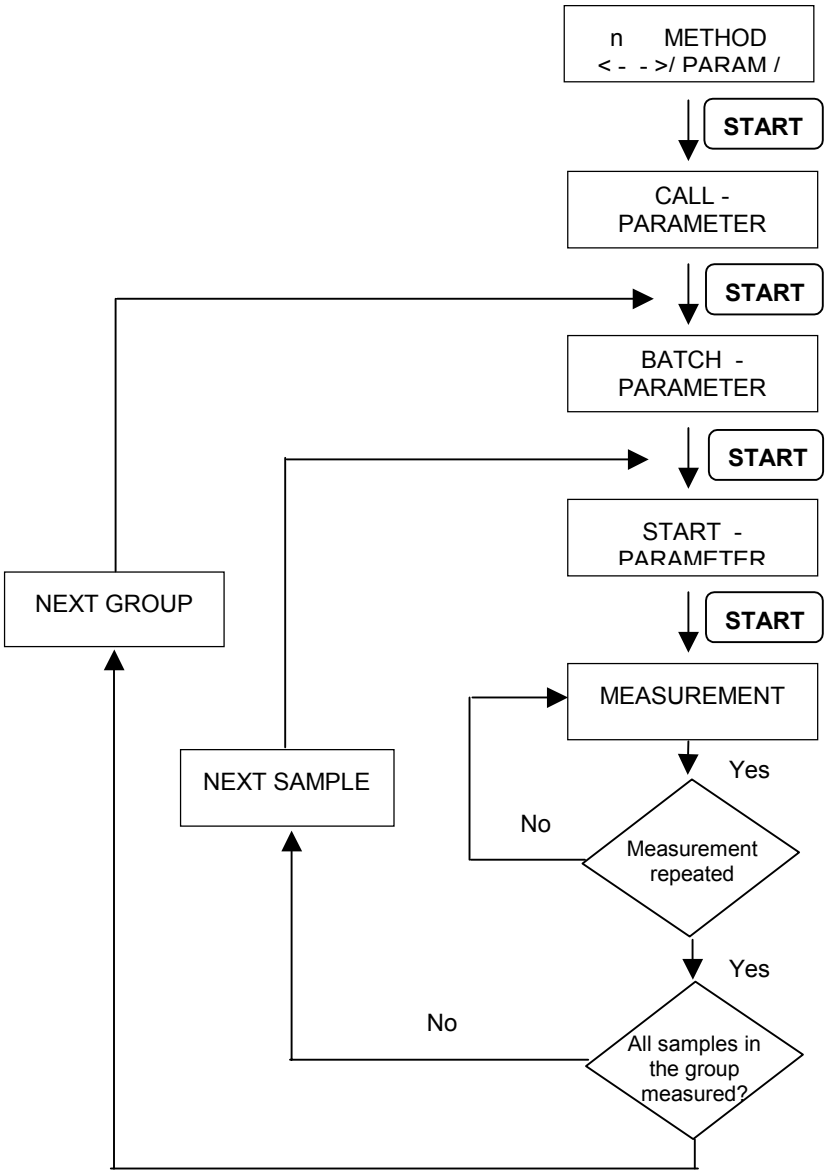


Figure 6 Flow diagram of a typical method procedure

Single Wavelength Measurements

Select a **TIME DRIVE** method to measure a sample at one wavelength over a defined period of time.

For Enzyme Activity methods, see *Enzyme Kinetics* on page 226.

Procedure

1. Select the **TIME DRIVE** method.

   The following table lists typical TIME DRIVE parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	ORDINATE MODE	A
3	WAVELENGTH	500.00 nm	11	FACTOR	1.0
14	RESPONSE	0.5 s	15	LAMP	UV + Vis
16	AUTOZERO	YES	38	ACCESSORY	MANUAL
18	SAMPLES/BATCH	0	19	FIRST SAMPLE #	1
21	CYCLES	1	22	CYCLE-TIME	0.10 min
25	GRAPHICS PLOT	YES	26	ORD.MAX	1.000 A
27	ORD.MIN	0.000 A	28	SCALE	20 nm/min
29	GRID	YES	32	PRINT DATA	NO

35 AUTO METHOD NO	**36 OPER ID . . . . . .**
37 SAMPLE ID	

* Only available with Lambda 40.

When a particular parameter is set to **YES** the extra parameters required automatically appear in their correct order.

For example, when **GRAPHICS PLOT** is **YES**, the extra parameters **ORD MAX, ORD MIN, SCALE** and **GRID** appear.

When **GRAPHICS PLOT** is **NO**, the extra parameters **ORD MAX**, **ORD MIN**, **SCALE** and **GRID** no longer appear.

2. If necessary, change the method parameters.

3. Press [START] to start the measurement.

4. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

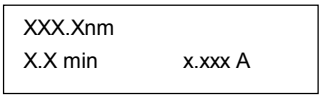
   OR
   If the display is:

   ```
   TIMEDRIVE          SAMPL 1
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

5. Continue to insert samples when asked until they have all been measured.
   For additional information, see *Replot* on page 79.

Example of the Display Shown During the Measurement



Xxx nm: Wavelength.

.xxx A Measured value; ordinate as selected.

Xxx min Time; units as selected.

C:xx Repeat measurement cycles still to be performed. This appears on the top right, when cycles > 1.

Printout

The result is printed out at the end of each sample analysis.

Scanning a Spectrum

Select a **SCAN** method to scan and record a spectrum of the sample.

Procedure

1. Select the desired **SCAN** method.

   The following table lists typical **SCAN** parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	ORDINATE MODE	A
3	WAV. MAX	1100.00 nm	4	WAV MIN	190.0 nm
13	SPEED	960 nm/min	14	SMOOTH	2 nm
15	LAMP	UV + Vis	16	AUTOZERO	YES
38	ACCESSORY	MANUAL	18	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1	21	CYCLES	1
22	CYCLE TIME	0.1 min	25	GRAPHICS PLOT	YES
26	ORD.MAX	1.000 A	27	ORD.MIN	0.000 A
28	SCALE	50.0 nm/cm	29	GRID	YES
30	OVERLAY	NO	32	PRINT DATA	NO
35	AUTO METHOD	NO	36	OPER. ID	. . . . .
37	SAMPLE ID	. . . . .			

* Only available with Lambda 40.

When a particular parameter is set to **YES** the extra parameters required automatically appear in their correct order.

For example, when **GRAPHICS PLOT** is **YES**, the extra parameters **ORD MAX, ORD MIN, SCALE** and **GRID** appear.

When **GRAPHICS PLOT** is **NO**, the extra parameters **ORD MAX**, **ORD MIN**, **SCALE** and **GRID** no longer appear.

2. If necessary, modify the method parameters.

3. Press [START] to start the method.

4. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

   OR
   If the display is:

   ```
   TIMEDRIVE          SAMPL 1
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

5. Continue to insert samples when asked until they have all been measured.
   For additional information, see *Replot* on page 79.

Example of the Display Shown During the Measurement

```
SCAN          SMPL 1
XXX nm        x.xxx A
```

Xxx nm: Wavelength.

.xxx A Measured value; ordinate as selected.

C:xx Repeat measurement cycles still to be performed. This appears on the top right, when cycles > 1.

Printout

The result is printed out at the end of each sample analysis: numerical data follow at the end of the analysis.

Measurement at Several Wavelengths

Select a wavelength program (**WAVELENGTH PROG**) method to measure a sample at several different wavelengths.

Procedure

1. Select the desired **WAVELENGTH PROG** method.

   The following table lists typical **WAVELENGTH PROG** parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	ORDINATE MODE	A
2	# WAVELENGTH	3	3	WAV. 1	459.9 nm
3	WAV. 2	418.5 nm	3	WAV. 3	360.0 nm
11	FACTOR 1	1.0	11	FACTOR 2	1.0
11	FACTOR 3	1.0	14	RESPONSE	0.5 S
15	LAMP	UV+Vis	16	AUTOZERO	YES
38	ACCESSORY	MANUAL	18	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1	21	CYCLES	1
22	CYCLE TIME	0.10 min	25	GRAPHICS PLOT	YES
26	ORD. MAX	1.000 A	27	ORD. MIN	0.000 A
28	SCALE	20 nm/min	29	GRID	YES

32	**PRINT DATA**	**YES**	**35**	**AUTO METHOD**	**NO**
36	**OPER. ID**	**.....**	**37**	**SAMPLE ID**	**.....**

***** Only available with Lambda 40.

When a particular parameter is set to **YES** the extra parameters required automatically appear in their correct order. When **GRAPHICS PLOT** is **YES**, the extra parameters **ORD MAX, ORD MIN, SCALE** and **GRID** appear.

When **GRAPHICS PLOT** is **NO**, the extra parameters **ORD MAX**, **ORD MIN**, **SCALE** and **GRID** no longer appear.

2. If necessary, modify the method parameters.

3. Press [START] to start the measurement.

4. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

   OR
   If the display is:

   ```
   WAVPROG          SAMPL 1
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

5. Continue to insert samples when asked until they have all been measured.
   For additional information, see *Replot* on page 79.

Example of the Display Shown During the Measurement

WAVPROG	SMPL 1
Xxx nm	x.xxx A

xxx nm: Wavelength.

.xxx A Measured value; ordinate as selected.

CYC:xx Repeat measurement cycles still to be performed. This appears on the top right, when cycles > 1.

Printout

The graphic result is printed out at the end of each sample analysis.

Concentration Determination

You use **CONCENTRATION 1** and **CONCENTRATION 2** methods to determine the sample concentration.

Using **CONCENTRATION** methods, you first establish a calibration curve and then measure the sample concentration.

The instrument calculates the calibration curve from the corrected or uncorrected values at defined wavelengths via the peak heights (**CONCENTRATION 1**), or the peak areas (**CONCENTRATION 2**), or the 2nd derivative (**CONCENTRATION 2**) of the spectrum.

CONCENTRATION 1 Method (Peak heights)

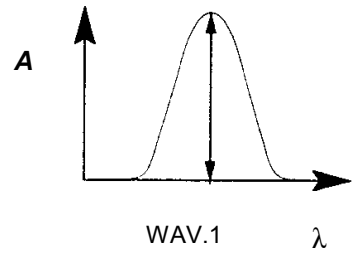
Summary of the procedure for creating a **CONCENTRATION 1** method:

- Determine the measurement wavelength(s) (see *Determining the Measurement Wavelength(s)* on page 91).
- Create a **CONCENTRATION 1** method (see *Creating a Method* on page 93).
- Establish a calibration curve using references (see *Establishing the Calibration Curve* on page 94).
- Measure the sample (see *Measuring the Sample* on page 95).

Determining the Measurement Wavelength(s)

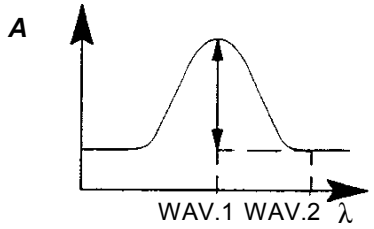
To determine the wavelengths:

1. Record the spectrum of the sample (see *Scanning a Spectrum* on page 85).
2. Select a strong peak and note the wavelength at its absorbance maximum (**WAV. 1**).



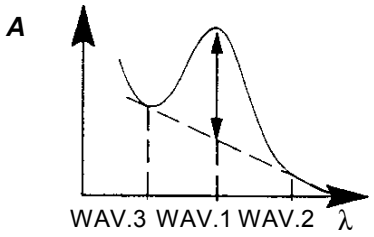
Select the type of baseline correction required:

3. With a straight but offset baseline, select a second wavelength at the baseline minimum (**WAV. 2**).



OR

With a sloping baseline, select a wavelength at the beginning and at the end of a peak (**WAV. 2** and **WAV. 3**).



Creating a Method

1. Create a new **CONCENTRATION 1** method (see *Creating a New Method* on page 57).

   The following table lists typical **CONCENTRATION 1** parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	MODE	1 WAVELENGTH
3	WAV. 1	500.0 nm	2	# OF REFS	3
6	CONC UNIT	µg/mL	7	REF 1	1.0 µg/mL
7	REF 2	2.0 µg/mL	7	REF 3	3.0 µg/mL
9	REFS.	NEW	8	VALUE 1	0.1
8	VALUE 2	0.2	8	VALUE 3	0.3
10	CUR FIT	LINEAR	11	FACTOR	1.0
12	DIVISOR	1.0	14	RESPONSE	1 s
15	LAMP	UV+Vis	16	AUTOZERO	YES
38	ACCESSORY	MANUAL	18	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1	21	CYCLES	1
22	CYCLE-TIME	0.01 min	32	PRINT DATA	YES
25	PLOT REFS	NO	26	PRINT REFS	YES

35 AUTO METHOD NO	**36 OPER. ID .....**
37 SAMPLE ID	

* Only available with Lambda 40.

2. Modify the parameters as required, using the wavelengths determined above.
   Once a method is created you can save it and use it for the same analysis when required without having to redetermine the wavelength.

Establishing the Calibration Curve

1. Press [START] to start the measurement.

2. Check the display.
   If the display is:

```
AUTOZERO
    START,STOP
```

   Insert cell(s) containing a blank solution and press [START]

   OR
   If the display is:

```
REF n                [XXX]
START,STOP,AUTOZERO
```

   Insert a cell containing the sample solution and press [START].

3. Insert the references in sequence when asked.

   When they have all been measured, the instrument prints out the calibration curve and the results.

   You can now amend the calibration curve (see *Processing the Calibration Curve (CONCENTRATION Methods)* on page 103) if required.

   You can use previously established calibration curves, or reference values (see **REFS** in *Parameter Numbers and Descriptions*, page 169).

Measuring the Sample

1. Press [START] to start the measurement.

2. Check the display.

   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

   OR

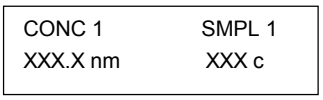
   If the display is:

   ```
   CONC 1              SMPL 1
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

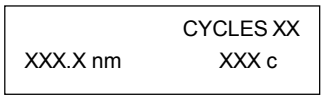
3. Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement



XXX nm: Wavelength.

xxx c: Result; units as selected



CYCLES:XX Repeat measurement cycles still to be performed. This appears on the top right, when cycles > 1.

Printout

If **PLOT REFERENCES** and **PRINT DATA** are set to **YES**, the calibration curve and results are printed out.

CONCENTRATION 2 Method (Peak Areas, 2nd Derivative)

Summary of the procedure for creating a **CONCENTRATION 2** method:

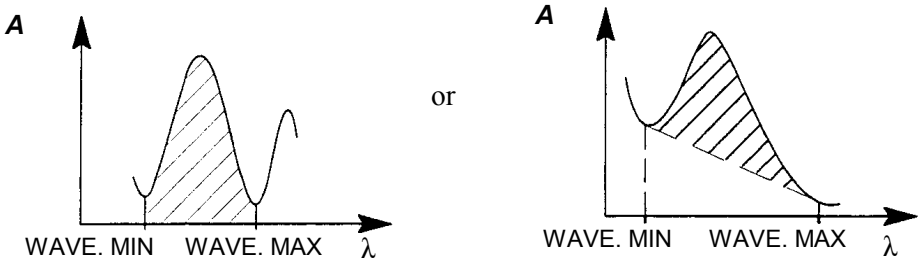
- Determine the measurement wavelength(s) (see *Determining the Measurement Wavelengths (Peak areas)* on page 96).
- Determine the threshold value (2nd derivative) (see *Determining the Measurement Wavelengths (2nd derivative)* on page 97).
- Create a **CONCENTRATION 2** method (see *Creating a Method* on page 99).
- Establish a calibration curve using references (see *Establishing the Calibration Curve* on page 101).
- Measure the sample (see *Measuring the Sample* on page 102).

Determining the Measurement Wavelengths (Peak areas)

To determine the wavelengths:

1. Record the spectrum of the sample (see *Scanning a Spectrum* on page85).

2. Select a strong peak and note the wavelength at its start (**WAVE. MAX**) and end (**WAVE. MIN**).



Determining the Measurement Wavelengths (2nd derivative)

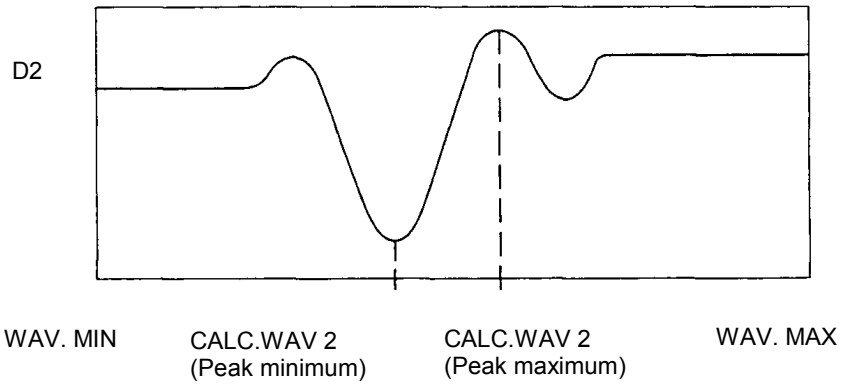
To determine the wavelengths:

1. Record the spectrum of the sample (see *Scanning a Spectrum* on page 85).

2. Select a strong peak and note the wavelength at its start (**WAV. MAX**) and end (**WAV. MIN**).

3. Record the spectrum of the same sample using the 2nd derivative (**D2** mode) over the wavelength range determined in step 2 above.

4. From the 2nd derivative spectrum determine the wavelength at the peak maximum and peak minimum.
   Use these values for **CALC. WAV 1** and **CALC. WAV 2**.

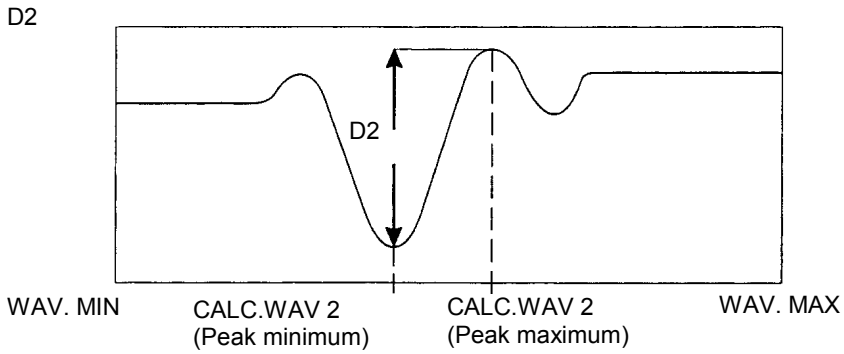


Determining the Threshold Value (2nd derivative)

To determine the threshold value:

1. Record the spectrum of the most dilute reference solution using the 2nd derivative (**D2** mode) over the wavelength range determined in step 2 or *Determining the measurement wavelengths (2nd derivative)* above.

2. Determine the value **D2** of this spectrum.
   D2 is the height from peak maximum to peak maximum.



3. Select a value for the threshold parameter somewhat lower than this **D2** value.

Creating a Method

1. Create a new **CONCENTRATION 2** method (see *Creating a New Method on page* 57).

   The following table lists typical **CONCENTRATION 2** parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	MODE	PEAK AREA
3	WAV. MAX	600.0 nm	4	WAV. MIN	500.0 nm
2	# OF REFS	3	6	CONC UNIT	C
7	REF 1	1.0 C	7	REF 2	2.0 C
7	REF 3	3.0 C	9	REFS	NEW
8	VALUE 1	0.1	8	VALUE 2	0.2
8	VALUE 3	0.3	10	CUR FIT	LINEAR
11	FACTOR	1.0	12	DIVISOR	1.0
13	SPEED	960 nm/min	14	SMOOTH	2 nm
15	LAMP	UV+Vis	16	AUTOZERO	YES
38	ACCESSORY	MANUAL	18	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1	21	CYCLES	1
22	CYCLE-TIME	0.1 min	32	PRINT DATA	YES
25	PLOT REFS	NO	26	PRINT REFS	YES
35	AUTO METHOD	NO	38	OPER. ID	. . . . .
37	SAMPLE ID	. . . . .			

* Only available with Lambda 40.

2. Modify the parameters as required, using the wavelengths and threshold values determined above.

   Once a method is created you can save it and use it for the same analysis when required without having to redetermine the values.

Establishing the Calibration Curve

1. Press [START] to start the measurement.

2. Check the display.

   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

   OR

   If the display is:

   ```
   REF n                [XXX]
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

3. Insert the references in sequence when asked.

   When they have all been measured, the instrument prints out the calibration curve and the results.

   You can now amend the calibration curve (see *Processing the Calibration Curve (CONCENTRATION Methods)* on page 103) if required.

   You can use previously established calibration curves, or reference values (see **REFS** in *Parameter Numbers and Descriptions* on page 169).

Measuring the Sample

1. Press [START] to start the measurement.
2. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution and press [START]

   OR
   If the display is:

   ```
   CONC 2              SMPL 1
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].
3. Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

```
CONC 2              SMPL 1
XXX.X nm            XXX c
```

xxx nm: Wavelength.

xxx c: Result; units as selected

```
                  CYCLES XX
XXX.X nm            XXX c
```

CYCLES:XX Repeat measurement cycles still to be performed. This appears on the top right, when cycles > 1.

Printout

If **PLOT REFERENCES** and **PRINT DATA** are set to **YES**, the calibration curve and results are printed out.

Processing the Calibration Curve (CONCENTRATION Methods)

Changing the type of Curve Fit

The type of calibration curve fit (linear or quadratic) can be altered without having to carry out additional measurements. The procedure is as follows:

1. Select **REFS = OLD**

   OR

   CUR FIT, as required.
   This modifies the method.

2. Press [START].
   The new curve fit is calculated on already available data.

Remeasuring the Reference Solution

Should a measuring point lie outside the calibration curve and need to be remeasured, proceed as follows:

1. Select **REFS = NEW**.

2. Press [START].
   You will be asked for first reference solution.

3. Enter the number of the reference to be remeasured, and press [ENTER].

   ```
   REF n                [XXX]
   START,STOP,AUTOZERO
   ```

4. Place the reference solution in the sample cell holder

5. Press [START].

6. Wait until the measurement is complete.
7. Press [STOP].
8. Set **REFS = OLD**.
9. Press [START].
   The new calibration curve is calculated with the new value.
10. If the new curve is satisfactory, measure the sample.

    OR

    If it is not acceptable, repeat the procedure.

Deleting a Point from the Calibration Curve

To delete such a point proceed as follows:

Linear Curve through Zero

1. In the method parameters, set **REF n = 0.000** and **VALUE n = 0.000**.
   n is the number of reference solutions to be deleted.
2. Press [ENTER] to confirm the changes.

Non linear curves, and curves with intercept

1. In the method parameters, note the ordinate value and the concentration value of the last point.
2. Replace the ordinate value and the concentration value of the point to be deleted (**REF n**) with the values noted in step 1 above.
3. Reduce the value for **# OF REFS** by 1.
4. Press [ENTER] to confirm the changes.

Enzyme Kinetics

Select an **ENZYME** method for enzyme kinetic measurements.

NOTE: *Enzyme activity is strongly dependent on temperature. Thus, the following should be taken into account:*

- *All measurements should be carried out at a constant temperature. You can use the temperature sensor (B0185227) for monitoring the temperature.*
- *All solutions and essential instrument accessories, especially cells and cell holders, should be thermostatted prior to use.*

Procedure

1. Select the appropriate **ENZYME** method.

   The following table lists typical **ENZYME** parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	3	WAVELENGTH	340.0 nm
14	RESPONSE	0.5 s	15	LAMP	UV+Vis
20	TIME UNIT	min	9	CALCULATE	REGRESSION
22	TOTAL TIME	1.0 min	21	INTERVAL	0.2 min
24	LAG TIME	0.0 min	11	ENZ.FACTOR	1.0
10	DIL. FACTOR	1.0	12	DIVISOR	1.0
7	BLANK	0.0	6	ENZ.UNITS	U/L
16	AUTOZERO	YES	14	SAMPLES/BATCH	0
19	FIRST SAMPLE #	1	25	GRAPHICS PLOT	YES
26	ORD.MAX	1.000 A	27	ORD.MIN	0.000 A
28	SCALE	20 mm/min	29	GRID	YES
32	PRINT DATA	ALL	34	POSTRUN KIN.	YES
35	AUTO METHOD	YES	36	OPER. ID	.....
37	SAMPLE ID	.....			

* Only available with Lambda 40.

2. Modify the method as required.

3. If necessary, determine the blank value of the reaction (see below) and enter the value in the parameter **BLANK**.

4. Press [START].
   This starts the measurement.

5. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution (distilled water) and press [START]

   OR
   If the display is:

   ```
   ENZYME              SMPL 1
   START,STOP,AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

6. Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

```
                    XXX nm
XXX nm              XXX A
```

XXX nm: Wavelength.

XXX min: Time.

XXX A: Measured value.

The display shown when using a cell changer:

```
XºC         n          C:001
XXX min     XXX A
```

XºC Temperature.

n: Cell location.

C:001: Cyclic number.

XXX min: Interval time.

XXX A Measured value.

Printout

The results are printed out at the end of the analysis.

Determining the Blank Value

Determine the blank value of the reaction as follows:

1. Select the appropriate **ENZYME** method.
2. Set parameter **BLANK = 0.0** in the method.
3. Carry out a measurement according to your procedure using a cell filled with redistilled water in place of the sample.
4. Enter the result of the measurement in the parameter **BLANK**.

Substrate Kinetics

Select a **SUBSTRATE KIN** method for substrate kinetic measurements.

Manual Procedure

1. Select the appropriate **SUBSTRATE KIN** method.

   The following table lists typical **SUBSTRATE KIN** parameters in the order (left to right) in which they appear.

   See *Parameter Numbers and Descriptions* on page 169for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	3	WAVELENGTH	340.0 nm
14	RESPONSE	0.5 s	15	LAMP	UV+Vis
20	TIME UNIT	min	23	DELAY TIME	0.0 min
22	END TIME	3.0 min	21	CREEPING CYCLE	0
11	CONC FACTOR	1.0	10	DIL.FACTOR	1.0
12	DIVISOR	1.0	7	BLANK	0.0
6	CONC UNIT	C	16	AUTOZERO	YES
18	SAMPLES/BATCH	0	19	FIRST SAMPLE #	1
25	GRAPHICS PLOT	YES	26	ORD.MAX	1.000 A
27	ORD.MIN	0.000 A	28	SCALE	20 mm/min
29	GRID	YES	32	PRINT DATA	ALL
34	POSTRUN KIN	YES	35	AUTO METHOD	YES
36	OPER.ID	.....	37	SAMPLE ID	.....

* Only available with Lambda 40.

2. Modify the method as required.

3. If necessary, determine the blank value of the reaction (see below) and enter the value in the parameter **BLANK**.

4. Press [START].
   This starts the measurement.

5. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   Insert cell(s) containing a blank solution (distilled water) and press [START]

   OR
   If the display is:

   ```
   SUBSTRATE        SMPL 1
         START,STOP
   ```

   Place solutions (with the exception of enzyme solution) in a cell and mix.

   Place the cell in the sample cell holder and press [START].

6. Allow equilibrium time (delay time) to elapse.

   ```
   DELAY TIME       XXX nm
   XXX min          XXX A
   ```

7. Add the enzyme solution and mix.

   ```
   - WAIT -         SAMPLE 1
         START,STOP
   ```

8. Press [START].

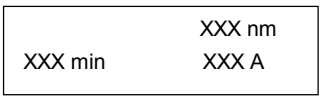
Procedure with a Cell Changer

Analysis is performed analogous to manual operation, the essential difference being that instead of one cell several can be used for measurement in the one operation.

The procedure is as follows:

1. Once the equilibrium time has elapsed (prior to addition of enzyme), the absorbance in each cell is measured automatically.
2. After adding enzyme, only location 1 is measured to follow the course of the reaction.
3. Once the reaction is complete the absorbance in all the remaining cells is measured.
4. Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

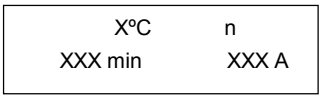


XXX nm: Wavelength.

XXX min: Time.

XXX A: Measured value.

The display shown when using a cell changer:



XºC Temperature.

n: Cell location.

XXX min: Interval time.

XXX A Measured value.

Printout

The results are printed out at the end of the analysis.

Determining the Blank Value

Determine the blank value of the reaction as follows:

1. Select the appropriate **SUBSTRATE** method.
2. Set parameter **BLANK = 0.0** in the method.
3. Carry out a measurement according to your procedure using a cell filled with redistilled water in place of the sample.
4. Enter the result of the measurement in the parameter **BLANK**.

Recalculation of Results with POSTRUN KIN

You can recalculate the results from **ENZYME** and **SUBSTRATE** methods using the **POSTRUN KIN** parameter.

When you set the **POSTRUN KIN** parameter to **YES** the following parameters in the corresponding method can be identified.

ENZYME method: **LAG** time and **TOTAL TIME**.

SUBSTRATE method: **END TIME** (manual operation only).

NOTE: *The time set for the* **LAG TIME** *must be smaller than the* **TOTAL TIME**.

NOTE: *If a cell changer is being used, make sure that both* **LAG TIME** *and* **TOTAL TIME** *are whole multiples of the* **INTERVAL** *time.*

Proceed as follows to calculate the results:

1. Create a method with **POSTRUN KIN = YES**.

2. Carry out a measurement (see *Enzyme Kinetics* on page 105 or *Substrate Kinetics* on page 109).
   At the end of the measurement **POSTRUN KIN** appears on the display.

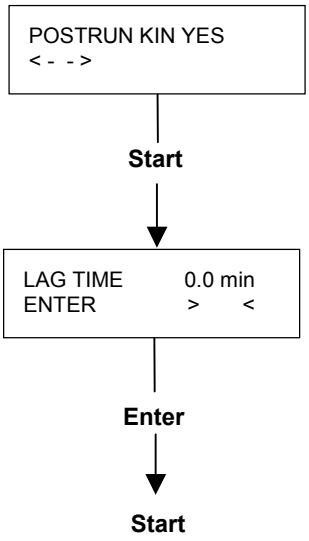
3. Press [START] to recalculate results.

   OR

   If the results are not to be recalculated, select **NO** using the arrow keys and press [START].

4. Enter new values as required for the parameter displayed and press [ENTER].

5. Press [START] to continue.



Methods for Quantitative Analysis of Oligonucleotides

Select an **OLIGOQUANT 1** method for quantitative analysis of oligonucleotides up to 50 bases long, and to calculate the theoretical melting point.

Select an **OLIGOQUANT 2** method for quantitative analysis of oligonucleotides longer than 50 bases, and to calculate the theoretical melting point.

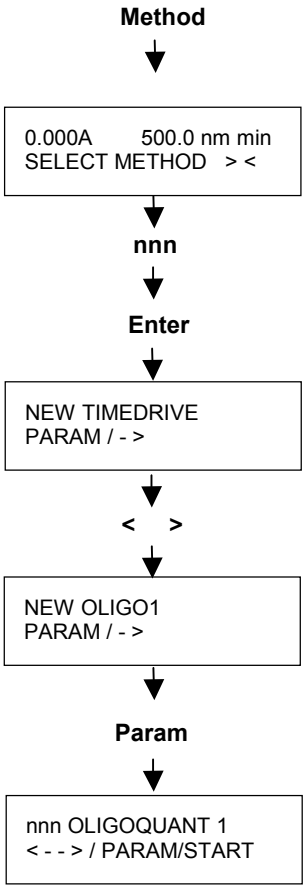
You create an **OLIGOQUANT** method as follows:

1. Press [METHOD].
2. Enter a method number **nnn** not previously used.
3. Press [ENTER].
   The first method of the **NEW METHOD** level appears.
4. Use the arrow keys to select the required method type (**Oligoquant 1** or **Oligoquant 2**).
5. Press [PARAM] to confirm the creation of the new method.

   OR

   Press [STOP] to cancel.

6. Modify the new method as required.



Procedure

1. Select the appropriate **OLIGOQUANT** method (see *Oligoquant Parameter Tables* on page 118).

2. If necessary, modify the method parameters.

3. Press [START].
   This starts the measurement.

4. Check the display.
   If the display is:

   ```
   AUTOZERO
       START,STOP
   ```

   OR
   If the display is:

   ```
   OLIGO n          SMPL 1
   START,STOP, AUTOZERO
   ```

   Insert a cell containing the sample solution and press [START].

5. Continue to insert samples when asked until they have all been measured.

Example of the Display Shown During the Measurement

```
OLIGO n          SMPL 1
XXX nm           XXX A
```

XXX nm: Wavelength.

XXX A: Measured value; ordinate as selected.

Printout

Graphics are printed out during the measurement process; numerical data follow at the end of the analysis.

Oligoquant Parameter Tables

The following table lists typical **OLIGOQUANT 1** parameters in the order (left to right) in which they appear.

See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	ORDINATE MODE	A†
2	# WAVELENGTHS	1†	3	WAV. 1	260.0 nm†
11	FACTOR 1	1.0	59	PATHLENGTH	1.0 cm
60	SEQUENCE LENGTH	20	61	SEQ.	1
61	SEQ	2	65	CHANGE CONSTANTS	NO
76	TM CALCULATION	NO	14	RESPONSE	1 s
15	LAMP	UV+Vis	16	AUTOZERO	NO
18	SAMPLES/BATCH	0	19	FIRST SAMPLE #	1
21	CYCLES	1	22	CYCLE TIME	0.01 min
25	GRAPHICS PLOT	NO	32	PRINT DATA	YES
35	AUTO METHOD	NO	36	OPER. ID	.....
37	SAMPLE ID	.....			

* Only available with Lambda 40.

† Do not change the value for this parameter otherwise you will get the wrong result.

Once the **OLIGOQUANT 1** method is created, the method parameters can be edited.

The base sequence must be entered from base 5′ to 3′.

Use the numeric keys according to the table below to enter the base sequence.

Base	Description	Key
N	Any base	8
A	Adenine	7
C	Cytosine	4
G	Guanine	1
T	Thiamine	0

The following table lists typical **OLIGOQUANT 2** parameters in the order (left to right) in which they appear.

See *Parameter Numbers and Descriptions* on page 169 for a detailed description of each parameter.

No.	Parameter	Value	No.	Parameter	Value
17	SLIT*	2.0 nm	1	ORDINATE MODE	A†
2	# WAVELENGTHS	1†	3	WAV. 1	260.0 nm†
11	FACTOR 1	1.0	59	PATHLENGTH	1.0 cm
60	NUMBER OF dA	0	60	NUMBER OF dC	0
60	NUMBER OF dG	0	60	NUMBER OF dT	0
60	NUMBER OF N	0	65	CHNGE CONSTANTS	NO
76	TM CALCULATION	NO	14	RESPONSE	0.5 S
15	LAMP	UV+Vis	16	AUTOZERO	YES
18	SAMPLES/BATCH	0	19	FIRST SAMPLE #	1
21	CYCLES	1	22	CYCLE TIME	0.01 min
25	GRAPHICS PLOT	NO	32	PRINT DATA	YES
35	AUTO METHOD	NO	36	OPER. ID	.....
37	SAMPLE ID	.....			

* Only available with Lambda 40.

† Do not change the value for this parameter otherwise you will get the wrong result.

Date/Time

1. Select the **DATE/TIME** method (**900**).
2. Press [PARAM].
3. Press [PARAM] again.
4. Use the arrow keys to select the realtime or the internal clock.
5. Press [PARAM] again.
6. Use the arrow keys to select the day.
7. Press [PARAM] again.
8. Type in the date (year, month, day - **960429**) using the numeric keys.
9. Press [ENTER] to confirm the entry.
10. Press [PARAM] again.
11. Type in the time (hours, minutes - **1430**) using the numeric keys.
12. Press [START] to activate the clock.

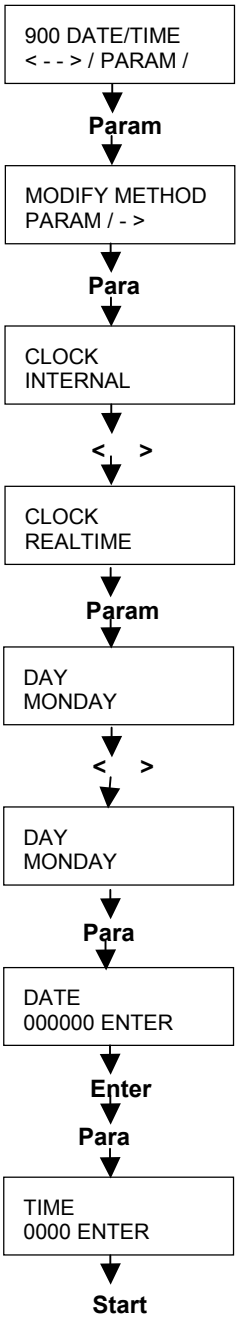
    OR

    Press [STOP] to cancel.

The realtime clock need only be set once, and has the following functions: day (Monday, Tuesday . . .), date (yymmdd) and time (hhmm), and continues working when the instrument is switched off.

The internal clock is limited to the following functions: date (yymmdd) and time (hhmm), and counts from the time the spectrometer is switched on.

The internal clock must be reset to actual time after each switch on.


900 DATE/TIME
< - - > / PARAM /
Param
MODIFY METHOD
PARAM / - >
Para
CLOCK
INTERNAL
< >
CLOCK
REALTIME
Param
DAY
MONDAY
< >
DAY
MONDAY
Para
DATE
000000 ENTER
Enter
Para
TIME
0000 ENTER
Start

Wakeup

You can use **WAKEUP** to set the spectrometer to switch on the lamps to warm up before the start of the working day.

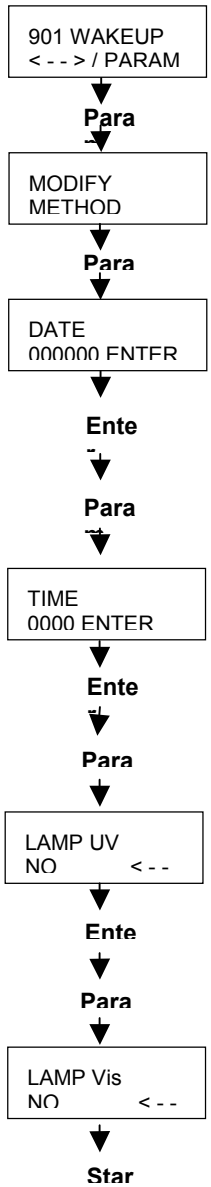
1. Select the **WAKEUP** method (**901**).
2. Press [PARAM].
3. Press [PARAM] again.
4. Type in the 'wakeup' date (year, month, day - **960429**)using the numeric keys.
5. Press [ENTER] to confirm the entry.
6. Press [PARAM] again.
7. Type in the 'wakeup' time (hours, minutes - **0655**) using the numeric keys.
8. Press [ENTER] to confirm the entry.
9. Press [PARAM] again.
10. Use the arrow keys [< - - >] to select UV lamp **On** or **Off**.
11. Press [ENTER] to confirm the entry.
12. Press [PARAM] again.
13. Use the arrow keys [< - - >] to select Vis lamp **On** or **Off**.
14. Press [START] to activate the wakeup method.

    OR

    Press [STOP] to cancel.

When this method is activated the lamps are switched off and are then switched on again at the preselected **WAKEUP** time.

To exit the **WAKEUP** method press [STOP] and the display returns to standby. Both lamps go on.



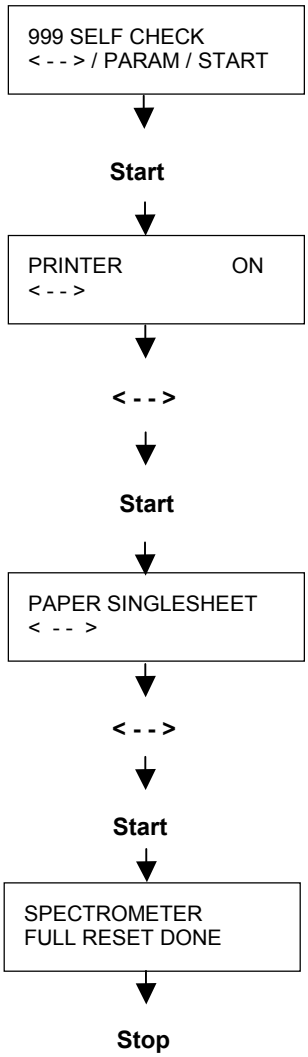
Self Check

The spectrometer calibrates the signals from the optics, and prints a report at the end. We recommend you do a self check at regular intervals.

1. Make sure that the beam path is not obstructed.
2. Select the **SELF CHECK** method (**999**).
3. Press [START] to activate the self test.
   The spectrometer then asks you to select the printer type you are using.
4. Use [< - - >] to select your printer type.
5. Press [START] to confirm the selection and move to the next parameter.
   Depending on the type of printer selected the spectrometer may ask you to select the type of paper you are using; Z-fold or single sheet.
6. Use [< - - >] to select your paper type.
7. Press [START] to confirm the selection and start the checks.
   When the checks are completed the spectrometer display shows **SPECTROMETER FULL RESET DONE.**
8. Press [STOP] to print the report and return to standby.

If there are any **FAIL** results in the report then repeat the test.

If you have any further enquiries contact your PerkinElmer office.


999 SELF CHECK
< - - > / PARAM / START
Start
PRINTER ON
< - - >
< - - >
Start
PAPER SINGLESHEET
< - - >
< - - >
Start
SPECTROMETER
FULL RESET DONE
Stop

Operating with Accessories 7

Operating with Accessories

General

Accessories are components, or instruments, that are installed or connected in the sample compartment, or otherwise connected to the spectrometer. For some of these accessories parameters have to be taken into account in the methods.

The accessories described below have parameters in the various methods.

Accessories

Samples can be applied either manually or with the help of a number of accessories.

The following accessories are currently available:

Changers	5 Cell Changer 6 Cell Changer 8 Cell Changer 9 Cell Changer 13 Cell Changer
Linear Transporter	8 cell changer; or gel plate reader
Sippers	Vacuum Sipper or Peristaltic Sipper
Auto samplers	AS-90/91

Requirements for Operation with Accessories

The following preconditions must be fulfilled in order to operate with accessories:

- The accessory in use must be properly selected in the accessory parameter on the appropriate method.

- The connector panel for the accessory in question must be installed in the spectrometer. Some accessories, such as Peltier or Temperature sensor, also require an accessory board.

The various circuit boards and connector panels are described in the *Installation and Maintenance Guide*.

Using Methods with Accessories

You must set the parameter, **ACCESSORY**, in the method to the appropriate accessory being used (see *Parameter Numbers and Descriptions* on page 169).

NOTE: *You can simulate operation with an accessory by setting the* **ACCESSORY** *parameter in the method to the accessory required. However, with cell changers only one sample can be measured.*

Simulation of Accessories

You can simulate operation with an accessory without the accessory being connected.

Proceed as follows to set up a method to simulate operation with an accessory.

1. Switch on the spectrometer in **SuperUser** mode (see *SuperUser* on page 207).
2. Select the **CONFIGURATION** branch.
3. Select method **7 ACCESSORY CONFIG**.
4. Select **ACCESSORY = YES**.
   This modifies the method.
5. Press [START].
   This stores the changes and activates the accessory mode.
6. Switch off the spectrometer.
   This deactivates the **SuperUser** mode.
7. Wait about two minutes to allow the lamps to cool down.
8. Switch on the spectrometer.
9. Set up the method as required.

Running Methods with an Accessory

When using methods with accessories, the spectrometer automatically presents the necessary actions.

Display	Description
AUTOZERO IN CELL 1 START,STOP	Insert blank at location 1 of the cell changer and then press [START].
SAMPLES INTO 9-CELL START,STOP,AUTOZERO	Insert samples in the cell changer, and then press [START].
AUTOZERO SIPPER ACCESSORY START	Press [START] on the Sipper.
SAMPLE 1 SIPPER ACCESSORY START	Press [START] on the Sipper (sample measurement).

Autozero with Cell Changers

The locations used for sample measurement depend on the tagging and option chosen for the **AUTOZERO** parameter.

Not all locations can be used for sample measurement, as shown in the following table.

AUTOZERO Tagging	Procedure	Locations			
		Blank solution	Sample solution	AUTOZERO =YES	AUTOZERO =NO
FIX/CALL	Insert solutions according to display	1		**1 . . . n**	**1 . . . n**
START	When selecting an autozero, first insert all solutions and then select YES or NO and press [START]	1		**2 . . . n**	**2 . . . n**
BATCH	If no autozero is selected, AUTOZERO is only selectable in the appropriate method. Thus, on demand to insert the sample, first insert all solutions and then start the first measurement.	1		**2 . . . n**	**1 . . . n**

CONCENTRATION Methods with Cell Changers

Please note the following when using CONCENTRATION methods::

When AUTOZERO = YES has been selected, an autozero is carried out once the method has been started, irrespective of whether the parameter has been tagged or not.

- Insert the reference solutions in sequence when asked.
- Always start at location 1.

Accessory Parameters

Parameter	Description
General	
ACCESSORY	Select the accessory.
MANUAL	Operation with standard cell holder, no accessories.
CELL	For cell changers, including: 5 Cell Changer, 6 Cell Changer, 8 Cell Changer, 9 Cell Changer, 13 Cell Changer.
SIPPER†	For Vacuum Sipper or Peristaltic Sipper.
AS 90/91*	For autosamplers AS-90 and AS-91.
LINTRANS	For linear transporter.

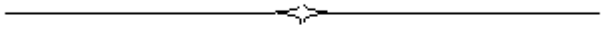
† If SUBSTRATE methods are used, Sipper operation is not possible.
* Autosampler operation is not possible when using ENZYME or SUBSTRATE methods.

Parameter	Description
Cell Changer (5 Cell, 6 Cell, 8 Cell, 9 Cell)	
CELL 1-n	The locations at which measurements are to take place. Enter the number of the locations and press [ENTER]. If measurement is to be carried out at locations 2 and 5 only, enter **25** Therefore: If **AUTOZERO = YES** has been selected, location 1 is used for background correction, irrespective of the locations selected as described above. If **AUTOZERO = YES** has been selected, place the blank at location 1 and the sample solutions from location 2. If **AUTOZERO = NO** has been selected, all locations can be used for sample measurement.

Parameter	Description
Cell changer (13 Cell Changer)	
CELL 1-7 **-CELL 8-13**	The locations at which measurements are to take place. When CELL 1-7 shows, the numbers 1 to 7 represent sample locations 1 to 7. When CELL 8-13 shows, the numbers 1 to 6 represent sample locations 8 to 13. If none of the locations is to be used, enter **0**. Press [ENTER] to confirm the location numbers. If measurement is to be carried out at locations 2 and 5 only, enter **25** and then press [ENTER] (CELL 1-7), and enter **0** and then press [ENTER] (CELL 8-13). If **AUTOZERO = YES** has been selected, place the blank solution at location 1 and the sample solutions from location 2. If **AUTOZERO = NO** has been selected, all locations can be used for sample measurement. Example 1: CELL 1-7 = 246 CELL 8-14 = 0 — Measurement will take place at locations 2, 4 and 6; the rest will not be used Example 2: — Measurement will take place at locations 5 to 13. CELL 1-7 = 567 CELL 8-13 = 123456

Parameter	Description
STIRRER	Switches the magnetic stirrer on and off. Option: YES NO Select with arrow key. If the magnetic stirrer has been switched on, place a small magnetic stirring bar in each of the cells. The arrangement is such that whilst measurement is taking place in the one cell the following cell will be stirred.
SAMPL. TIME	Sample aspiration time in seconds for the sipper. Range: 0.1 to 99.9 Enter value and confirm by pressing [ENTER].
DELAY TIME	Delay between the end of the aspiration process and the start of the measurement. Range: 0.0 to 99.9 Enter value and confirm by pressing [ENTER].
AUTO PURGE	Switches the autopurge function on and off. Option: YES NO Select with arrow keys.
RETURN (peristaltic sipper only)	Pump in reverse direction after sample measurement. Option: YES NO Select with arrow keys.

Parameter	Description
RETURN TIME (peristaltic sipper only)	To enter the reversed flow time for the optimization. Range: 0.0 to 99.9 Enter value and confirm by pressing [ENTER].
SIPPER	To select the sipper accessory. Further information about operation is described in the user documentation provided with the sipper.
AS 90/91	To select the autosampler accessory. Further information about operation is described in the user documentation provided with the autosampler.
LINTRANS	To select the linear transporter accessory. Further information about operation is described in the user documentation provided with the linear transporter.



8 *Care*

Care

WARNING

Unauthorized Adjustments and Servicing

Do not attempt to make adjustments, replacements or repairs to this instrument except as described in the accompanying User Documentation.

Only a PerkinElmer service representative or similarly trained and authorized person should be permitted to service the instrument.

Daily Care

- Do not leave samples, particularly those given to fuming or evaporation, in the sample compartment for longer than necessary.

- If any type of sample handling system is installed and portions of it are left in the sample compartment (such as a sipper and flowcell), make certain that the system is cleaned at the end of the working day.

  Generally, such systems should be filled with deionized water when left overnight.

- Immediately clean all spilled materials from the affected area and wipe it dry with lintless paper or cloth.

  If you have to wipe the sample compartment windows, make sure you do not introduce scratches.

  Sample windows are optical components and you should handle them in the sampe way as high quality cells.

CAUTION

Risk of damage to Spectrometer

Take care not to spill liquids onto the spectrometer. Expensive damage can result to the optics or electronics if liquids are spilled and run inside the instrument or onto the keyboard.

Use and Care of Cells

Cell Handling

- Only hold cells by non-optical surfaces, such as the matt finish surfaces.
- Always wipe the optical surfaces of cells dry and free of fingermarks, using a soft cloth or cleaning tissue, just before placing them in the cell holder.
- Protect cells from scratches, and never permit them to rub against one another or against other hard surfaces.
- Avoid abrasive, corrosive or stain-producing cleaning agents, and make certain that the exposed surfaces of cells are optically clean.
- When measuring cold solutions, always bear in mind that condensation can form on the optical surfaces.
- Make certain no bubbles cling to the inner surfaces of the cell, particularly when handling cold solutions.
- For maximum precision and accuracy, calibrate and test with cells of the same type, and always insert cells into the holders with the same orientation.

Pressure Buildup in Cells

- Only fill the cell so full that the liquid meniscus is just above the radiation beam. The remaining air space in the cell is then adequate to compensate for any slight increase in pressure in the cell during routine operation.
- If, for analytical reasons, it is necessary to fill the cell completely, insert the stopper only lightly so that the liquid in the cell has a chance to expand.
- Do not insert a stopper forcefully into a completely filled cell since this is likely to cause the cell to burst.

- When working at higher temperatures, use a drilled stopper (0.4 mm hole) to allow for expansion in the cell.

Sample Compartment Windows

- Generally, the windows should be installed at all times.
- The windows are optical components and require the same care and handling as cells.
- You can remove windows to clean them.

  They are held in place by a magnetic frame.

  Windows are most suitably cleaned by wiping them with a soft lint free cloth moistened with ethanol.

Analytical Notes 9

Autozero

The type of autozero depends on the method type selected.

In methods with a fixed wavelength as **TIME DRIVE**, **WAVELENGTH PROGRAM**, **CONCENTRATION 1**, the displayed measurement value for absorbance is set to 0, for transmission to 100%, at the measurement wavelength (this is called an autozero).

You can use the [AUTOZERO] key to perform a manual autozero in fixed wavelength methods (see *Manual Autozero* on page 42).

In methods with measurement over a wavelength range such as **SCAN**, **CONCENTRATION 2**, a background correction is performed over the selected wavelength range. A background correction can only be performed in a method.

The ordinate mode of the last used method always appears on the display. To change from absorbance to transmittance or vice versa, select a **TIME DRIVE** method, and then select the desired ordinate mode.

An autozero, or background correction, must be performed:

- At the start of a new method,
- When the wavelength is changed,
- When the wavelength range is extended,
- When the scan speed is changed,
- Each time the solvent is changed.

To perform an autozero, or background correction, place cells with a blank solution (or as directed in the method) in the sample cell holder and reference cell holder.

Unusual Samples

If a sample is chemically stable and undergoes no physical or chemical change other than to absorb incident radiation, errors in photometric values should not be caused by the sample. Many samples are not stable, and special consideration must be given to them.

Volatile Samples

Some liquid samples are so volatile that their concentration can change while recording is in progress. If this occurs, the resulting data will lack reproducibility.

If you are analyzing volatile samples, use stoppered cells to prevent this problem.

Samples not Governed by the Beer-Lambert Law

Quantitative analyses utilizing the absorption of spectral radiation are based on the Beer-Lambert law which states that the absorption is proportional to the concentration of the analyte.

The law can be expressed in the form

$\boldsymbol{A} = \varepsilon\, c\, d$

Where:

A is absorbance

ε is molar absorption coefficient

c is molar concentration

d is thickness through which the radiation is transmitted

This law is mostly true for dilute solutions, but at higher concentrations a plot of absorbance aga-inst concentration will be nonlinear for a number of reasons.

The absorption characteristics of a sample can be changed during sample preparation, depending on the amount of reagent added for color development and so on. For details, refer to reference books covering these subjects.

Temperature has an influence to a greater or lesser degree on the absorption characteristics of a sample. You should check this effect if non-repeatable results are obtained.

If you are measuring temperature-dependent samples, either wait until temperature equilibrium has been attained or use a thermostatted cell or cell holder.

Chemically Reactive Samples

If a reaction takes place in the cell between the sample material and the solvent, spectral data based on that sample cannot always be expected to have sufficient reliability or repeatability.

For samples of this type, use a quantitative method that takes advantage of the change in transmittance with time at a fixed wavelength.

For details, refer to reference books covering this specific subject.

Photoactive Samples

Some samples are known to be photoactive in that they fluoresce upon absorbing radiation. Since a small portion of the fluorescent radiation will be measured by the detector, a higher apparent transmittance will often result.

Samples are also known that undergo photochemical reactions as they absorb radiation. With such samples, which are mostly biochemical, lack of reproducibility will characterize the resultant data.

Other Sample Properties

Samples that are polarizing in nature, or have a double index of refraction, are often difficult to measure accurately. The emerging monochromatic radiation is slightly polarized due to having been refracted.

Thin-film samples also pose a problem since optical interferences may develop, causing a regular interference pattern to be superimposed on the spectral curve.

Solvent Properties

The solvent should meet the following requirements:

- It should dissolve the sample without reacting with it.
- The radiation absorption in the scanning region should be low.
  High absorption by the blank reduces the reference energy, thus increasing noise.
- Evaporation should be fairly low at ambient temperature.

In general, aromatic compounds exhibit high absorption in the UV region and hence are not suitable as solvents for measurements in this region.

Water is virtually the only useful solvent below 195 nm, but it must be freed from oxygen to attain best transmission.

Whenever you are going to use a solvent with unknown absorption characteristics, scan its spectrum first to determine whether it is suitable.

The lower wavelength limits of a number of commonly used solvents are presented in the following table.

The lower limit has been defined as that wavelength at which 10 mm of pure solvent has a transmission of 10%.

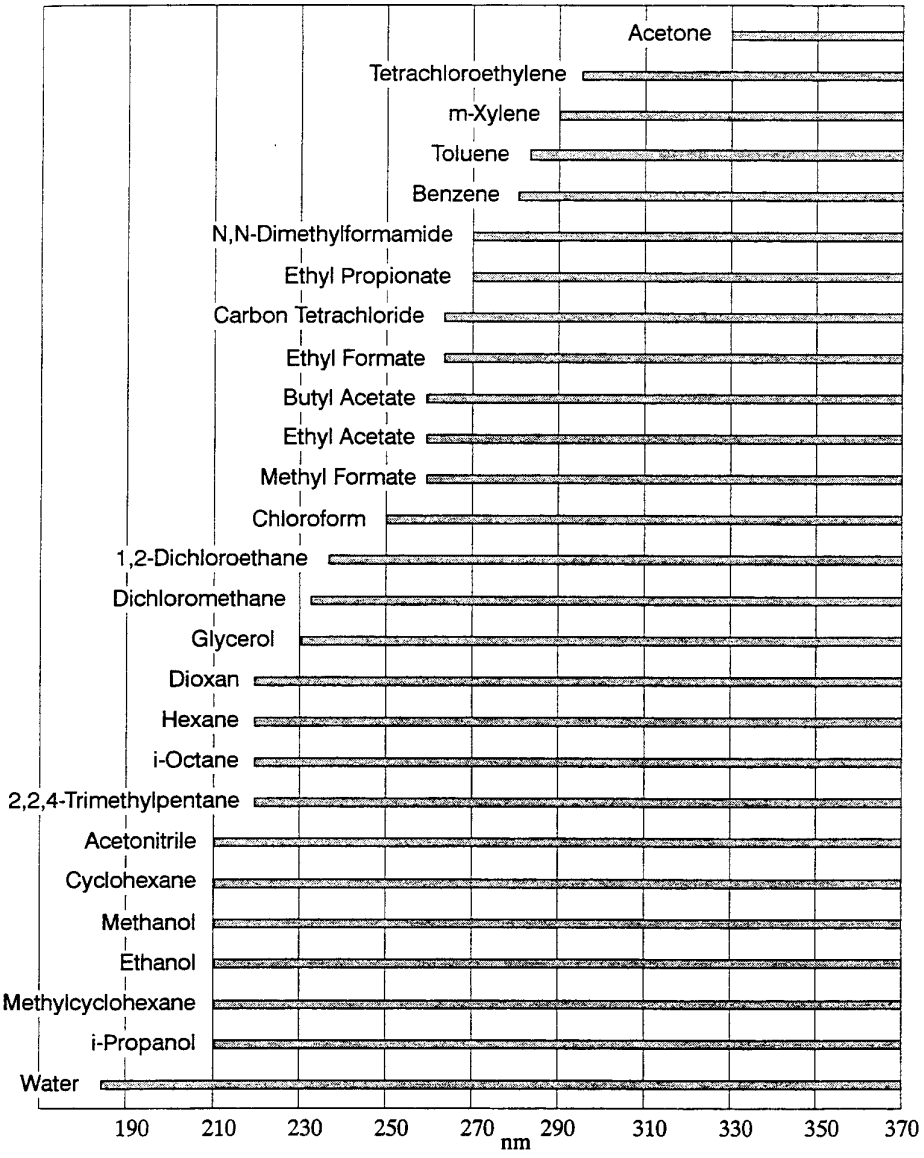


Figure 7 Lower Wavelength Limits of Solvents

Error Messages 10

Error Messages

If an error occurs during the operation of the spectrometer, an error message is shown on the display or is printed out (if a printer is connected).

Error Messages Shown on the Display

Errors remain displayed until they are deleted. To delete, press [PARAM].

Error	Meaning
RANGE ERROR: xxxx.x – xxxx.x	The value entered is outside the displayed range. Press [PARAM] and enter a value within the range shown.
PARAMETER XX: DOES NOT EXIST	The parameter **XX** does not exist. Press [PARAM] to continue.
PARAMETER XX: NOT USED	The parameter **XX** is not used, or is not active in this method. Press [PARAM] to continue.
HINT: INT.GREATER TOT.TIM	The Interval time is greater than the total time. This message appears with kinetic measurements. Select a shorter interval time.
HINT: SELECT INTERVAL TIME	Appears with enzyme methods with cell changers, if the interval time does not correlate with the measurement time. Select the **INTERVAL** time so that the **TOTAL TIME** can be divided by the **INTERVAL** time evenly.

Error	Meaning
PROBLEM: MARK NOT SET	**COPY FROM MARK** was selected without first initiating **MARK FOR COPY**. First use **MARK FOR COPY** and then select **COPY FROM MARK**.
PROBLEM: METHOD NOT FOUND	A method has been tagged **MARK FOR COPY** although the method in question is no longer available.
PROBLEM: ACCESSORY NOT INITIALIZED	This error is shown when the instrument gets no response from an attached accessory during startup. Check that the accessory is: - correctly connected - switched on - functioning normally
PROBLEM: METHOD NO. LIMITS 1-999	The method number entered is outside the displayed range. Enter a number within the range 1 to 999.
ERROR: LAST METHOD	An attempt was made to delete all methods. Retain at least one method in the memory otherwise the spectrometer cannot work.
PROBLEM: METHOD PROTECTED	An attempt was made to select a fully protected method. The protection has to be modified if the method is to be used.
DON'T PROTECT ALL METHODS	An attempt was made to **ALL** protect all the methods. Retain at least one method otherwise the spectrometer cannot work.

Error	Meaning
DON'T PROTECT ALL BRANCHES	An attempt was made to **ALL** protect all branches. Retain at least one branch otherwise the spectrometer cannot work.
PROBLEM: BRANCH WRITE PROTECT	An attempt was made to modify methods in a write-protected branch. To modify the method, alter the branch protection.
PROBLEM: DIRECTORY FULL	An attempt was made to store more than 200 methods. To create space for the new method, delete a method that is no longer required.
PROBLEM: MEMORY FULL	The available memory is insufficient to cope with the new method. To create space for the new method, delete a method that is no longer required.
ERROR: NO ENERGY	This error is shown when not enough energy is detected. Possible causes: - Beam is blocked in the sample compartment - Loose lamp connection - Lamp burnt out - Lamp(s) switched off - Defective detector

Error	Meaning
ERROR: NO ENERGY, UV LAMP	This error is shown when not enough energy is received from the UV lamp. Possible causes: - Beam is blocked in the sample compartment - UV lamp loose connection - UV lamp burnt out - UV lamp switched off - Defective detector
PROBLEM: NO ENERGY, VIS LAMP	This error is shown when not enough energy is received from the Vis lamp. Possible causes: - Beam is blocked in the sample compartment - Vis lamp loose connection - Vis lamp burnt out - Vis lamp switched off - Defective detector

Error	Meaning
PROBLEM: SYSTEM ERROR	This error is shown when the instrument operating software "crashes". A full reset is then automatically carried out.* After the instrument is reset one of the following messages is shown on the second line of the display: **BATTERY LOW** **TIMER FAIL** **RS232-IRQ FAIL** **TIMER-IRQ FAIL** Make a note of this message. Press [PARAM] to continue. If you cannot continue call your PerkinElmer office and inform them of the error message.
SPECTROMETER FULL RESET DONE	This error is shown after changing the instrument software, or after a full reset*, or when the spectrometer data is defect. Make a note of the steps you made leading up to this message. Press [PARAM] to continue. If you cannot continue call your PerkinElmer office and inform them of the error message and the steps you made leading up to the error.

* After a full reset all methods are erased.

Error	Meaning
DIALOG, FULL RESET DONE	This error is shown after changing the instrument software, or after a full reset*, or when the method memory is defect. Make a note of the steps you made leading up to this message. Press [PARAM] to continue. If you cannot continue call your PerkinElmer office and inform them of the error and the steps you made leading up to the error.
SPECTROMETER + DIALOG FULL RESET DONE	This error is shown after changing the instrument software, or after a full reset*. Make a note of the steps you made leading up to this message. Press [PARAM] to continue. If you cannot continue call your PerkinElmer office and inform them of the error and the steps you made leading up to the error.
BUS ERROR + a message	This error, plus a message, is shown when the instrument has an address error. Make a note of this message and the steps you made leading up to the error. Press [PARAM] to continue. If you cannot continue call your PerkinElmer office and inform them of the error, the error message and the steps you made leading up to the error.

* After a full reset all methods are erased.

Error Reports on the Printer

Error	Meaning
Baseline correction data do not fit. Start baseline correction	The background correction last carried out did not correlate to the method used. Carry out a new background correction with the proper method.
Cannot approximate calibration curve. Check references or change curve fit algorithm.	The calibration data deviate strongly from the curve form selected or not enough points were measured for the curve form selected. Check the references, select another curve form or carry out more measurements.
Cannot calculate delta absorbance. Because too few points read.	A kinetic method was interrupted with [STOP]. Too few points available to calculate delta absorbance.
Cannot calculate slope. Because too few points read.	A kinetic method was interrupted with [STOP]. Too few points available to calculate the slope.
End time out of limits. Change end time.	In recalculation, an out of limits value for **END TIME** was entered. Enter a lower **END TIME** value and repeat the procedure.

Error	Meaning
Lag or total time is not divisible by interval time (0.0).	In recalculation, the **LAG TIME** or **TOTAL TIME** entered was not divisible by the **INTERVAL** time. Enter a value for **LAG TIME** or **TOTAL TIME** that is fully divisible by the **INTERVAL** time.
Lag or total time out of limits. Change lag and/or total time.	In recalculation, a greater value for **LAG TIME** or **TOTAL TIME** was entered than the actual measuring time. Enter a lower value for **LAG TIME** or **TOTAL TIME**.
Lag time greater than total time. Change lag time?	In recalculation, a value for **LAG TIME** was entered that was greater than the measuring time. Enter a **LAG TIME** less than the **TOTAL TIME**.
More than one peak within wavelength limits. Change threshold or measurement wavelengths.	More than one peak identified within the selected measuring range. Change **WAV. MIN** and **WAV. MAX** or **THRESHOLD** so that only one peak is detected.
No peak detected. Change threshold.	The **THRESHOLD** is too high to detect peaks. Select a lower value for **THRESHOLD**.

Error	Meaning
No points stored. **Start measurement.**	The method was interrupted with [STOP] before data could be stored, for example, during the equilibrium time of a substrate method. Restart the method.
Two solutions to 1 ABS value. **Change curve fit algorithm.**	In a non-linear calibration curve two reference solutions exhibit the same absorbance value. Check the references, or change the concentration range, or change the curve fit algorithm.
Value not within valid limits. **Check references or change curve fit alogrithm.**	The measured sample concentration is outside the calibration range. Measure additional calibration solutions within the concentration range or change the curve fit algorithm.
Wavelength data do not fit. **Start background correction.**	Appears with **TIME DRIVE** methods if the wavelength has been modified since the last background correction. Perform a new background correction using the proper method.

Parameter Numbers and Descriptions 11

Parameter Numbers and Descriptions

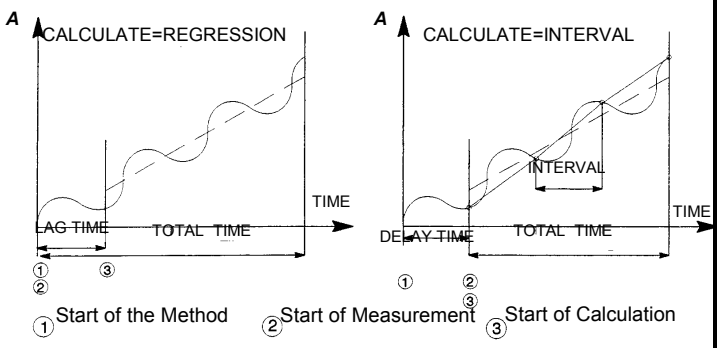
Parameter	N o .	Description
# OF BATCHES	**55**	To enter the number of batches to be analyzed. Range: 1 to 50 Appears with the autosampler accessory.
# MEASUREMENTS	**54**	To set the number of repeat sample measurements from each sample tube. Range: 1 to 9 Appears with the autosampler accessory.
# OF REFS.	**2**	Number of reference solutions used for the calibration. Range: 1 to 20
# WAVELENGTHS	**2**	Number of wavelengths at which measurements are made. Range: 1 to 20 If ordinate modes **RAT** and **DIF** are used, the number must be divisible by 2. If ordinate mode **COR** is used, the number must be divisible by 3. See also parameter Ordinate Mode.
ABS.COEF.dA	**71**	To set the molar absorption coefficient of the Adenine nucleotide. Deoxyadenosine monophosphate (dAMP). Range: 0 to 99999 Appears with the Oligoquant applications.

Parameter	No.	Description
ABS. COEFF.dC	**72**	To set the molar absorption coefficient of the Cytosine nucleotide. Deoxycytidine Monophosphate (dCMP). Range: 0 to 99999 Appears with the Oligoquant applications.
ABS.COEFF.dG	**73**	To set the molar absorption coefficient of the Guanine nucleotide. Deoxyguanosine monophosphate (dGMP). Range: 0 to 99999 Appears with the Oligoquant applications.
ABS.COEFF.dT	**74**	To set the molar absorption coefficient of the Thymine nucleotide. Deoxythymidine monophosphate (dTMP). Range: 0 to 99999 Appears with the Oligoquant applications.
ABS.COEFF.dN	**75**	To set the molar absorption coefficient of any nucleotide. Deoxynucleoside monophosphate (dNMP). N is used to represent any base. Range: 0 to 9999 Appears with the Oligoquant applications.
ACCESSORY	**38**	Lets the spectrometer recognize the accessory type connected. **MANUAL** is for operations without using an accessory, or for manual cell changers. **CELL** is for a cell changer. **SIPPER** is for a sipper.

Parameter	No.	Description
		AS 90/91 is for the AS-90/91 autosampler. **LINTRANS** is for the linear transporter. **NOTE:** *Sipper operation is not possible when using SUBSTRATE or ENZYME methods.* **NOTE:** *Autosampler operation is not possible when using ENZYME methods.*
AUTO METHOD	**35**	Prints method parameters prior to each method start. Options: **YES NO**
AUTO PURGE	**48**	Switches the autopurge function on and off. Options: **YES NO**
AUTOZERO	**16**	**Autozero.** Options: **YES NO** Without tagging, autozero should be carried out at the start of the method. If tagging has been carried out, autozero is offered: - At the start of the method (**CALL** tagging) - Prior to each sample batch (**BATCH** tagging) - Prior to each sample (**START** tagging) **Time Drive, Wavelength Prog.** Autozero (or setting the baseline to zero) is carried out at every wavelength. **Scan.** Background correction is carried out within the range **WAV.MAX** and **WAV.MIN**. The baseline is set to zero.

Parameter	N o .	Description
		Concentration 1, Concentration 2. If **AUTOZERO = YES** has been selected, autozero must be carried out prior to measurement of the first reference solution. Autozero should be carried out at wavelengths **WAVELENGTH 1-3**. That is, the baseline signal is set to zero at these points. **Substrate, Enzyme.** Autozero is carried out at the wavelength selected.
BLANK	**7**	Blank value. **Enzyme.** Blank value (units as selected for **ENZ UNIT**). Range: -9999.9 to 9999.9 The enzyme activity is calculated as follows: $a_{Enz} = a_{Total} - a_{Blank}$ **Substrate.** Blank value (units as selected for **CONC UNIT**). Range: -9999.9 to 9999.9. The substrate concentration is calculated as follows: $c_{Sub} - c_{Total} - c_{Blank}$
CALC. WAV 1	**5**	Wavelength in nm (used in **CONCENTRATION 2** methods for calculations using the 2nd order derivative only). Range: 190.0 to 1100.0, in steps of 0.1. The values must lie within the **WAV.MAX** and **WAV.MIN** values set. Enter the value at peak maximum for **CALC. WAV 1** (see

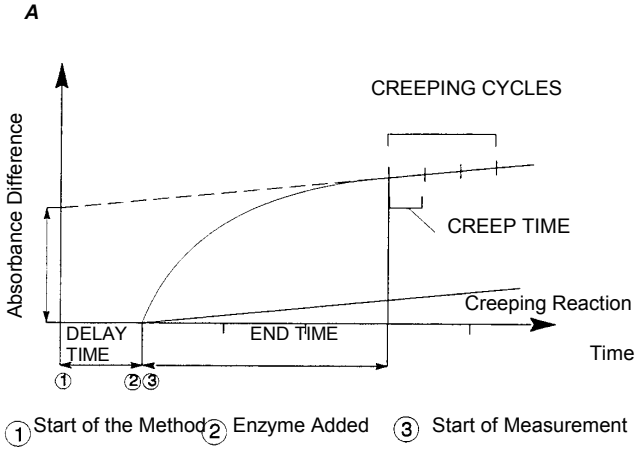
Parameter	No.	Description
		Determining the Threshold Value (2nd derivative) on page 98). In **MODE = DERIV 2 FIX,** the height of the derivative curve is measured at these wavelengths. In **MODE = DERIV 2 PEAK,** a derivative maximum or minimum is located around these wavelengths and the nearest one evaluated (see **Error! Reference source not found.**).
CALC. WAV 2 **CALC. WAV 2 (cont.)**	**5** **5**	Wavelength in nm (used in **CONCENTRATION 2** methods for calculations using the 2nd order derivative only). Range: 190.0 to 1100.0, in steps of 0.1. The values must lie within the **WAV. MAX** and **WAV. MIN** values set. Enter the value at peak minimum for **CALC. WAV 2** (see *Determining the Threshold Value (2nd derivative)* on page 98). In **MODE – DERIV 2 FIX**, the height of the derivative curve is measured at these wavelengths. In **MODE = DERIV 2 PEAK**, a derivative maximum or minimum is located around these wavelengths and the nearest one evaluated (see **Error! Reference source not found.**).
CALCULATE	**9**	Slope calculation mode: **REGRESSION:** The slope is calculated using all data points by means of linear regression. **INTERVAL:** The slope is calculated for each interval. The mean of all the slopes is then used for the calculation of enzyme activity. **NOTE:** *When using* **CALCULATE = INTERVAL** *please*

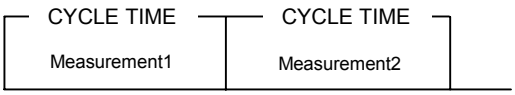
Parameter	N o .	Description
		bear in mind: instead of the parameter **LAG TIME**, *parameter* **DELAY TIME** *(equilibration time) appears. Measurement begins only when the* **DELAY TIME** *has elapsed. The* **TOTAL TIME** *must be a whole multiple of the* **INTERVAL** *time.* 
CELL 5	**39**	To enter the location of the cell, or cells, to be measured in the 5-cell changer.
CELL 6	**40**	To enter the location of the cell, or cells, to be measured in the 6-cell changer.
CELL 8	**43**	To enter the location of the cell, or cells, to be measured in the 8-cell changer.
CELL 9	**44**	To enter the location of the cell, or cells, to be measured in the 9-cell changer.
CELL 1-7	**41**	To enter the location (1 to 7) of the cell, or cells, to be measured in the 13-cell changer. Example:

Parameter	No.	Description
		Cell 1-7 = 2 4 6 **Cell 8-13 = 0** Measurement will take place at locations 2, 4 and 6; the rest will not be used.
CELL 8-13	**42**	To enter the location (8 to 13) of the cell, or cells, to be measured in the 13-cell changer. Example: Cell 1-7 = 5 6 7 Cell 8-13 = 1 2 3 4 5 6 Measurement will take place at locations 5 to 13.
CHNGE CONSTANTS	**65**	To change the molecular mass and molar absorption coefficient values of oligonucleotide bases. Option: **YES NO** Refer to the *Biochemical Application* manual.
CONC FACTOR	**11**	Concentration factor. Range: 0.00001 to 9999.9 **NOTE:** *The concentration factor is calculated as follows:* *Concentration Factor* = $\frac{V \times M}{d \times v \times 1000}$ *where:* V *is the volume of the total solution in the cell in mL* M *is the molar mass of the substrate in g/mol* *d is the pathlength in cm* *v is the volume of sample in mL* *1000 is the conversion factor for volume units in liters* *Depending on the procedure used, the molar absorption*

Parameter	No.	Description	
		coefficient may need to be taken into account.	
CONC UNIT	**6**	Concentration Unit, defines the concentration unit used for the analysis.	
		c	any concentration unit
		g/L	gram per liter
		mg/L	milligrams per milliliter
		mg/dL	milligrams per deciliter (Substrate methods only)
		µg	microgram
		µg/mL	micrograms per mililiter
		mol	mole
		mmol	millimole
		nmol	nanomole
		pmol	picomole
		ppm	parts per million (mg/kg)
		ppb	parts per billion (µg/kg)
		%	percent
		A1%	absorbance of a solution containing 1 g substance in 100 mL of solution, in a cell of 1 cm pathlength

Parameter	No.	Description
		APHA color number **OD** optical density (absorbance)
CREEPING CYCLE	**21**	Number of cycles after the reaction end time. Range: 0 (no further measurements after end point) 2 to 99 This parameter is used to compensate for creeping reactions: The spectrometer calculates the slope for each interval. If it remains constant, the substrate reaction is complete; the spectrometer will then determine the difference in absorbance for the substrate reaction. If the slope does not remain constant, two further measuring intervals are added.

Parameter	No.	Description
CREEP TIME	**24**	Duration of the measuring interval (units as selected for **TIME UNIT**). Appears only if **CREEP CYCLES** is 2 or more. Range: 0.1 to 999.9 
CUR FIT	**10**	Different types of calibration Curve Fit can be calculated by the spectrometer software. **LINEAR** — Used when the measured values vary linearly with the concentration, the curve passes through the origin. **LIN INTERC** — Used when the measured values vary linearly with the concentration, the curve has an intercept on the measured value axis to compensate for background interferences.

Parameter	No.	Description
		QUADRATIC Used when the measured values do not vary linearly with the concentration, the curve passes through the origin.
		QUAD INTERC Used when the measured values do not vary linearly with the concentration, the curve has an intercept on the measured value axis to compensate for background interferences.
CYCLE-TIME	**22**	The time in minutes between the start of one sample measurement to the start of the next sample measurement. Range: 0.002 to 999.9  Duration of the analysis = **CYCLES** x **CYCLE TIME** **NOTE:** When using accessories, set the **CYCLE TIME** longer than required for scanning the spectrum. This avoids time problems.
CYCLES	**21**	The number of times one sample is scanned or measured. Range: 0 to 99 **NOTE:** *If* **CYCLES** *is set to* **0***, the sample is scanned or measured continuously until you stop the method*.
DELAY TIME	**23** **47**	Equilibration time. (Only in the case of use with the cell holder or in manual operation with **CALCULATE = INTERVAL**). This is the time from the start of the method to the start of measurement (units as selected for **TIME UNIT**).

Parameter	No.	Description
		Range: 0.0 to 999.9 **NOTE:** *Measurement begins after equilibration time has elapsed.* **Substrate.** Once the equilibration time has elapsed, add the enzyme solution to the cell and mix. **Operating with a Sipper.** Delay between the end of the aspiration process and the start of the measurement. Range: 0 to 99.9
DIL. FACTOR	**10**	Dilution factor. The measured value is multiplied by the factor and the result displayed. Thus, concentration can be read off directly or a dilution taken into account. Range: 0.00001 to 9999.9
DIVISOR	**12**	Divisor. Range: 0.00001 to 999.9 The molar absorption coefficient value can be entered as divisor. Values can be obtained from the literature. **NOTE:** *If the absorption coefficient is already included in the* **ENZ FACTOR** *(enzyme factor), enter* **DIVISOR = 1**. **Enzyme.** Enzyme activity is automatically calculated as follows: A_{Enz} = enzyme factor x dilution factor x 1/divisor x d***A***/d*t* **Substrate.** Substrate concentration c_{Sub} is calculated as follows:

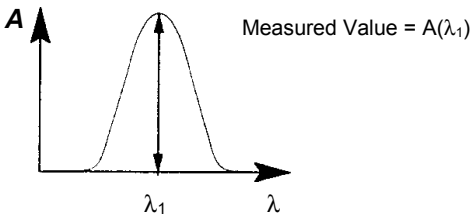
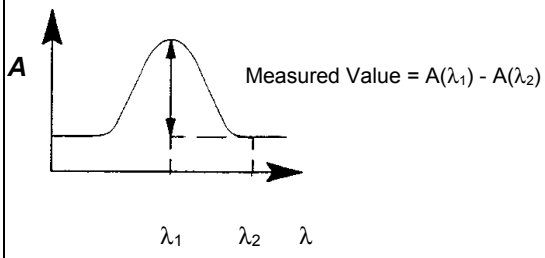
Parameter	No.	Description
		c_{Sub} = concentration factor x dilution factor x 1/divisor x ΔA Where ΔA = absorbance difference. **Concentration 1, Concentration 2.** The measured value is multiplied by the factor or divided by the divisor and the resultant value displayed. Thus, dilution procedures or differing masses can be taken into account. If a dedicated correction factor is to be used for each sample, select **FACTOR** or **DIVISOR** as a **START (TAG) PARAMETER** (see *Tagging a Parameter* on page 54). The factor or divisor can be entered immediately prior to each analysis. Example: A particular component of a powder is to be determined and displayed in mg/g. The calibration curve is compiled using pure substance in solution made up in mg/L. In order that the results can be displayed independently of the actual mass of powder used, the mass of the powder should be entered as the divisor. If the powder were dissolved in 0.25 L instead of in 1 L, an additional dilution factor of 0.25 should be entered. The results are then automatically calculated as follows: (0.25/mass of powder in g) x concentration in mg/L = concentration in mg/g.
END TIME	**22**	Time from the start of the reaction (addition of enzyme) to the end of the reaction (units as selected for **TIME UNIT**). Range: 0.1 to 999.9

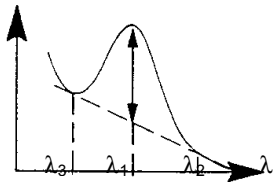
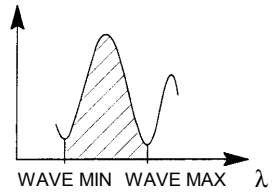
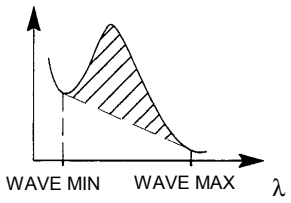
Parameter	No.	Description
ENZ. FACTOR	**11**	Enzyme factor. Range: 0.00001 to 9999.9 Calculate the enzyme factor as follows: Enzyme factor = *V/dv* Where: *V* is the volume of the total solution in the cell in mL *d* is the pathlength in cm *v* is the volume of sample in mL **NOTE:** *Depending on the procedure used, the molar absorption coefficient may need to be taken into account.*
ENZ UNIT	**6**	**Enzyme Unit.** **U/L** — Units per liter. **U/mL** — Units per milliliter. **mU/L** — Millinunits per liter. **U** — Units. **mU** — Milliunits. **mg/mL** — Milligrams per milliliter. - — Any unit. **Enzyme Unit U** — The amount of enzyme which catalyzes the conversion of 1 µmol (or micro-equivalent) of substrate per minute. The unit may appear in capitals on the display.

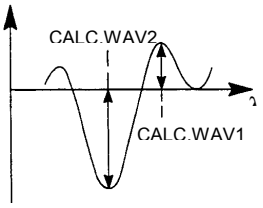
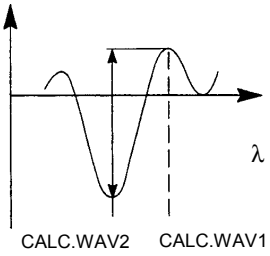
Parameter	No.	Description
FACTOR or **FACTOR n**	**11**	Factor. Range: 0.000001 to 9999.99 Factor (for each wavelength n). Range: -9999.9 to 9999.9 The measured value is multiplied by the factor and the result displayed. Thus, concentration can be read off directly or a dilution taken into account. If only the true measured value is to be shown, then choose **FACTOR** (or **FACTOR n**) **= 1**. If concentration units are to be read off directly, calculate the factor according to the Beer-Lambert law: $A = \varepsilon c d$ Where: A is the absorbance ε is the molar absorption coefficient c is the concentration of the sample d is the pathlength of the cell Thus, the concentration $c = A/(\varepsilon d)$ And the factor $f = 1/(\varepsilon d)$
FIRST SAMPLE #	**19**	Number of the first sample in the batch. All subsequent samples are automatically numbered consecutively.
FIRST TUBE n	**52**	The first sample tube location of the batch to be analysed. **n** is any location number up to the maximum location for the tray type.

Parameter	No.	Description
		Range: 1 to the maximum location number of the tray being used. Appears with the autosampler accessory.
GRAPHICS PLOT	**25**	Graphics printout. Option: **YES NO**
GRID	**29**	Graphics printout with grid. (valid only when **GRAPHICS PLOT = YES**) Option: **YES NO**
INTERVAL	**21**	Interval time (units as selected for **TIME UNIT**). Range: 0.1 to 999.9 When using **CALCULATE = REGRESSION**, the change in absorbance d*A*/d*t* is printed out. When using **CALCULATE = INTERVAL**, the slope is calculated for each interval. The mean of all slopes is then used for the calculation of the enzyme activity (see also **CALCULATE**). Operating with the Cell Changer: During the interval time the spectrometer measures once at each location.To do this you need to know the minimum measuring time t_{min}: $t_{min} = N \times (3 \times \text{RESPONSE} + x + 0.1)$ seconds where: N = the number of cells 3 x RESPONSE = measuring time per cell x = relocation time from cell to cell The interval time should always be greater than the required minimum time.

Parameter	No.	Description
LAG TIME	**24**	Lag time. This is the time from the start of the method to the start of calculation (units as selected for **TIME UNIT**). After this time a constant reaction rate should have been reached. Range: 0.0 to 999.9 Measurement begins with the start of the method. However, enzyme activity is only calculated from the end of the lag time. (Only when using **POSTRUN KIN = YES**, or manual operation together with **CALCULATE = REGRESSION**).
LAMP	**15**	Switched on lamps. UV: 190 nm to 326 nm VIS: 326 nm to 1100 nm UV/VIS: 190 nm to 1100 nm **NOTE:** *In order to preserve the UV lamp, switch off the lamp only at the end of the working day, and allow the lamp to cool off for at least 2 minutes before switching on again.*
LAST TUBE n	**52**	The last sample tube location of the batch to be analysed. **n** is any location number up to the maximum location for the tray type. Range: 1 to the maximum location number of the tray being used. Appears with the autosampler accessory.

Parameter	N o .	Description
LINE TYPE	**31**	Type of line used for the graphics printout (valid only when **GRAPHICS PLOT = YES**). **DASH1:** —————— **DASH2:** - - - - - - - - - - - - - - - **DASH3:** ..... **DASH4**: —·—·—·—·—·—·— **AUTO:** For each curve, a different type of line is used in the sequence **DASH1, DASH2, DASH3, DASH4, DASH1, … .**
MODE	**1**	**Concentration 1.** Sets the mode in which the measurements are made. **1 Wavelength; 2 Wavelength; 3 Wavelength** **1 Wavelength:** measuring absorbance at one wavelength.  **2 Wavelength:** correction for a sloping baseline by measuring absorbance at two wavelengths. 

Parameter	N o .	Description
		3 Wavelength: correction for a sloping baseline by measuring absorbance at three wavelengths. *A*  Measured Value = $A(\lambda_1) - \frac{(A(\lambda_2) - A(\lambda_3))(\lambda_1 - \lambda_3)}{\lambda_2 - \lambda_3} - A(\lambda_3)$ **1 Concentration 2.** Sets the mode in which the measurements are made. There are four possibilities: **TOTAL AREA** **PEAK AREA** **DERIV 2 FIX** **DERIV 2 PEAK** **TOTAL AREA:** the total peak area between wavelength maximum and wavelength minimum is calculated.  **PEAK AREA:** the peak area between wavelength maximum and wavelength minimum is calculated with a correction for the baseline drift. 

Parameter	N o .	Description
		DERIV 2 FIX: height of the 2nd order derivative curve at wavelengths **CALC.WAV2** and **CALC.WAV1** is measured.  **DERIV 2 PEAK:** height of the 2nd order derivative peak. The software locates the extreme values around wavelengths **CALC.WAV2** and **CALC.WAV1** and calculates the difference in height between these two points. Only values that have exceeded the set **THRESHOLD** are taken into account. This function can avoid errors that may occur through displacement of the spectrum. 
MOL.MASS dA	**66**	Sets the relative molecular mass of the Adenine nucleotide. Range: 0 to 99999 Appears with the Oligoquant methods.

Parameter	N o .	Description
MOL.MASS dC	**67**	Sets the realitve molecular mass of the Cytosine nucleotide. Range: 0 to 99999 Appears with the Oligoquant methods.
MOL.MASS dG	**68**	Sets the relative molecular mass of the Guanine nucleotide. Range: 0 to 99999 Appears with the Oligoquant methods.
MOL.MASS dT	**69**	Sets the relative molecular mass of the Thymine nucleotide. Range: 0 to 99999 Appears with the Oligoquant methods.
MOL.MASS N	**70**	Sets the relative molecular mass of the nucleotide. **N** is used to represent any base. Range: 0 to 99999 Appears with the Oligoquant methods.
NUMBER OF dA	**60**	Sets the number of Adenine nucleotides. Range: 0 to 999 Appears with the Oligoquant methods.
NUMBER of dC	**61**	Sets the number of Cytosine nucleotides. Range: 0 to 999 Appears with the Oligoquant methods.

Parameter	No.	Description
NUMBER of dG	**62**	Sets the number of Guanine nucleotides. Range: 0 to 999 Appears with the Oligoquant methods.
NUMBER of dC	**61**	Sets the number of Thymine nucleotides. Range: 0 to 999 Appears with the Oligoquant methods.
NUMBER OF N	**64**	Sets the number of bases. **N** is used to represent any base. Range: 0 to 999 Appears with the Oligoquant methods.
[OLIGO]	**78**	To enter the concentration of primer (DNA) in the solution. This is not necessarily the concentration in the cell. Used for the melting point calculation. Units are nmol/L. Range: 0.001 to 99999.9 Shows only when T_M (theoretical melting point) is **YES**. Refer to the *Biochemical Application* manual.
OPER.ID	**36**	User identification up to a maximum 8 characters. User identification appears on each printout.
ORD.MAX	**26**	Maximum ordinate scale range for graphics printout (valid only if **GRAPHICS PLOT = YES**). Range: -9999.9 to 9999.9 If the analytical value obtained is outside the set values, the latter should be changed. **NOTE:** *Derivative spectra can also have negative values.*

Parameter	No.	Description
ORD.MIN	**27**	Minimum ordinate scale range for graphics printout (valid only if **GRAPHICS PLOT = YES**). Range: -9999.9 to 9999.9 If the analytical value obtained is outside the set values, the latter should be changed. **NOTE:** *Derivative spectra can also have negative values.*
ORDINATE MODE	**1**	Ordinate Mode. **%T:** transmittance in percent **A:** absorbance **D1 to D4:** 1st to 4th derivatives of the spectrum (derivative spectra) **RAT:** absorbance ratio **DIF:** absorbance difference **COR:** Corrected Absorbance ratio **CONC:** concentration (see also **FACTOR**) Derivative modes D1 to D4 can be used to resolve overlapping peaks, to reduce interference and to enhance the fine structure of a particular peak. This facilitates the qualitative evaluation of spectra with overlapping peaks and the quantitative evaluation of spectra with undesired background absorption. The derived values obtained are multiplied by 10 for every degree of derivation in order to produce graphics that are easier to interpret. Resolution and noise increase with the degree of derivation. In general, the 2nd derivative is more helpful in this respect than the 1st: the resolution is better and the characteristic maximum of the signal is easy to recognize as a derivative minimum.

Parameter	N o .	Description
		Should the 1st or 2nd derivatives prove insufficient, the 3rd or 4th derivatives can be used, providing the noise level remains within acceptable limits. The parameters **SPEED** and **SMOOTH** influence the quality of derived spectra. In choosing parameter values, take the following into account: **SPEED**: guideline value = peak width in nm x 10. High scan speeds decrease resolution; low scan speeds increase noise. **SMOOTH**: in the case of derivation spectra, smoothing exerts a greater influence than in absorbance measurement. Smoothing should thus be kept to a minimum. Guideline value < peak width in nm x 0.5. Derivation is not possible with a degree of smoothing of 0, irrespective of the ordinate mode selected. Ratio, difference and corrected ratio (**RAT, DIF, COR**) modes are determined according to the equations below: $(\mathrm{RAT} = \frac{A_1}{A_2}, \frac{A_3}{A_4}, \ldots)$ $(\mathrm{DIF} = A_1 - A_2, A_3 - A_4, \ldots)$ $(\mathrm{COR} = \frac{A_1 - A_3}{A_2 - A_3}, \frac{A_4 - A_6}{A_5 - A_6}, \ldots)$ where subscript 1 stands for the first wavelength, subscript 2 stands for the second wavelength, and so on.

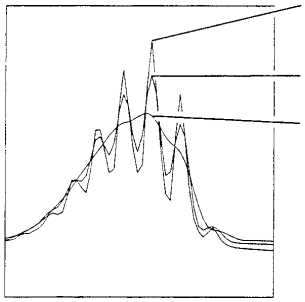
Parameter	No.	Description
OVERLAY	**30**	Prints spectra from the same batch onto the same graphics printout (valid only when **GRAPHICS PLOT = YES**). Option: **YES NO** Overlaying graphics printouts, functions only if the printer used has an automatic paper reverse function. If **CYCLES = 1**, all the results of a particular batch are printed out sequentially. This enables spectra to be more easily compared than if they are printed out separately.
PATHLENGTH	**59**	To enter the pathlength (cm) of the cell. The result is corrected for pathlength. Range: 0.001 to 10.0
PLOT REFS.	**25**	Prints out the calibration curve. Option: **YES NO**
POST RUN KIN.	**34**	Allows you to recalculate the results. Option: **YES NO** Enzyme: When **POSTRUN KIN = YES** is used, the results for a changed **TOTAL TIME** and **LAG TIME** can be recalculated. When using a cell changer, the values for **TOTAL TIME** and **LAG TIME** must be a whole multiple of the set **INTERVAL** time. Substrate: When **POSTRUN KIN = YES** is used, the results for a changed reaction **END TIME** can be recalculated. When using a cell changer no recalculation is possible.

Parameter	No.	Description
PRINT DATA	**32**	Prints out a table containing analytical data when activated. Option: **YES NO** Substrate Kinetics, Enzyme Kinetics: Option: **ALL FINAL NO** **ALL** All readings and the final calculated results are printed. **FINAL** Only the final calculated results are printed. **NO** No data printed.
PRINT REFS	**26**	Prints out data from the references when activated. Option: **YES NO**
READER		Read mode for the linear transporter. Option: **RANDOM CONT.** **RANDOM** Measurements at user defined positions. **CONT.** Continuous measurement over the entire 100 mm measurement range of the linear transporter.
REFS.	**9**	Reference solutions. Choice as to whether a calibration curve should be established or not at the start of a method. Option: **OLD NEW** **OLD** means you wish to use the "**OLD**" stored calibration curve. **NEW** means you wish to make a "**NEW**" calibration curve. If a calibration curve is to be used again, or if the values of the curve are to be entered directly, select

Parameter	No.	Description
		REFERENCES = OLD (see also **VALUE**). It is often useful to tag **REFERENCES**. For example: To tag a reference as a **CALL** parameter, select **REFERENCES = NEW** when selecting the first method and generate a calibration curve. At the next call-up, select **REFERENCES = OLD.** The available calibration curve is used and measurement can start immediately.
REF n	**7**	Concentration of reference solution n.
RESPONSE	**14**	Response time. Time constant in seconds (s). Option: **0.1**; **0.2**; **0.5**; **1**; **2**; **5**; **10** A large value for time constant gives good signal/noise ratios, but can cause undesirable smoothing of the curve, especially with rapidly altering signals. Thus the time constant should be kept as low as possible, but maintaining an acceptable signal/noise ratio. **NOTE:** *In analyses involving creeping reactions, the time constant set must be lower than the* **CREEP TIME** *set.* **NOTE:** *The time constant set must be lowe than the* **INTERVAL** *in order to calculate* d*A*/d*t (see* **INTERVAL***).*
RETURN	**79**	To operate the pump in the reverse direction to return the sample to the sample tube. Option: **YES NO** Appears with the peristaltic sipper accessory.
RETURN TIME		To set the amount of time for pumping in reverse. Range: 0.0 to 99.9

Parameter	No.	Description
[SALT]	**77**	To enter the salt concentration (monvalent cation concentration) of the oligonucleotide sample. Used for the melting point calculation. Units in mmol/L. Range: 0.1 to 999.9 Shows only when T_M (theoretical melting point) is YES. Refer to the *Biochemical Application* manual.
SAMPL. TIME	**46**	Aspiration time in seconds for sipper. Range: 0.0 to 99.9 Appears with the sipper accessory.
SAMPLE FLUSH	**51**	To flush the flowcell after each sample measurement. Option: **YES NO**
SAMPLE ID	**37**	Sample identification up to a maximum of 8 characters. Sample identification appears on every printout.
SAMPLES/BATCH	**18**	Number of samples per batch (does not appear when using the cell changer). If batch procedure is not used, enter **0**. Range: 0 to 999
SCALE	**28**	Abscissa scale in nm/cm or mm/min for graphics printout (valid only when **GRAPHICS PLOT = YES**). Options: **0.5**; **1**; **2**; **5**; **10**; **20**; **50**; **100** nm/cm; **1**; **2**; **5**; **10**; **20**; **50**; **100** mm/min

Parameter	No.	Description
SEQ.n	**61**	Sequence of the oligonucleotide bases. The sequence must be entered in order from base 5′ to 3′. Use the numeric keys according to the table below to enter the base sequence. Option: Base — Key **A** Adenine **7** **C** Cytosine **4** **G** Guinine **1** **T** Thymine **0** **N** any base **8**
SEQUENCE LENGTH	**60**	Length of the oligonucleotide base sequence. Range: **2** to **50**
SLIT	**17**	Required slit width in nm. Options: **0.5**, **1**, **2**, **4** **NOTE:** *Only shows with Lambda 40 spectrometer.*
SMOOTH	**14**	Smoothing according to Savitsky-Golay in nm. The acceptable level of smoothing is dependent on the scan speed. **SPEED**: Up to 960 nm/min — 1920 nm/min — 2880 nm/min **SMOOTH**: 0, 2, 3, 4 6, 8, 10 nm — 0, 4, 6, 8, 10 nm — 0, 6, 8, 10 nm

Parameter	No.	Description
		Guideline: 0.5 x peak width of lowest peak.  Smooth 2 nm, corresponds to ½ Peak width. Spectrum quality not compromised. Smooth 6 nm. Spectrum quality poor. Smooth value is 10 nm. Benzene spectrum not recognizable. Smoothing influences resolution and noise: an increase in smoothing decreases the noise, but resolution suffers. A too high degree of smoothing tends to average out the values and the fine structures of the spectrum disappear. If smoothing is too low, spectral fine structures may be hidden by noise. If an unacceptable value is selected, an appropriate indication is displayed. If then no other value is substituted, the spectrometer will automatically continue to operate at smoothing 0. **NOTE:** *Derivative spectra cannot be scanned at smoothing 0.* Concentration methods: Select a smoothing factor that is as low as possible so that the peak is smoothed as little as possible. The value selected should depend on the level of noise.

Parameter	N o .	Description
SPEED	**13**	Scanning speed in nm/min. Select the scanning speed depending on the type of sample and the desired resolution. The following can be used as a general rule: - for narrow peaks, select a low scanning speed and low degree of smoothing in order to limit the noise level. - for broader peaks, higher scanning speeds can be selected. - For derivative spectra, observe the comments given under **ORDINATE MODE**. Guideline values: Overview spectra 2880; 1920 nm/min Broad peaks 960; 480; 240 nm/min Solid and liquid samples 120; 60; 30 nm/min Gaseous samples, spectra with higher resolution expanded spectra 30; 15; 7.5 nm/min
STATISTIC	**56**	To select the statistic calculation function. To calculate and print out the average, the standard deviation, and relative standard deviation for each sample (repeat measurements), or sample group (batch measuremnts) when **YES**. Option: **YES NO** Appears with the autosampler accessory. Not available with all applications.

Parameter	N o .	Description
STIRRER	**49**	Switches magnetic stirrer on and off. Option: **ON OFF** If magnetic stirring has been switched on, place a small magnetic stirring bar in each of the cells. The arrangement is such that whilst meaurement is taking place in one cell, the following cell is stirred. Appears with the 13-cell changer accessory.
TEMP CHECK	**50**	Temperature measurement. Option: **YES NO** If **TEMP CHECK = YES** has been selected, a temperature sensor (B0185227) must be installed. The temperature measured in the cell is included in the printout. If a temperature sensor has not been installed, select **TEMP CHECK = NO.**
TEMPERATURE	**51**	To enter the required temperature in °C (only in combination with **TEMP CHECK = YES**). Range: –; 0.0 to 150.0 Measurement begins as soon as the temperature in the cell has reached the required temperature +0.1 °C. When – (minus) is selected the temperature is only registered and printed out together with the anaytical results. Operation with a Peltier cell holder: To enter the required temperature of the Peltier cell holder. Range: 15.0 to 45.0

Parameter	No.	Description
THRESHOLD	**33**	Scan: Threshold value for the printout of analytical data (valid only when **PRINT DATA = YES**). Range: 0.0 to 9999 Only data above the given threshold value is printed out. Concentration2: Threshold value for **MODE = DERIV PEAK 2**. Only values above the given threshold value will be recognized as peaks.
TIME UNIT	**20**	Units for all subsequent time parameters. min: minutes s: seconds
TIME CALCULATION	**76**	Calculates the theoretical melting point T_M. Refer to the *Biochemical Application* Manual.
TOTAL TIME	**22**	Total time from the start of the method (or end of **DELAY TIME**) to the end of the measurement (units as selected for **TIME UNIT**). Range: 0.1 to 999.9 Select the measuring time so that the end of the measurement is still within the linear portion of the curve. Manual operation: For **CALCULATE = REGRESSION**, the time is that between the start of the method and end of the measurement. For **CALCULATE = INTERVAL**, the time is that between the end of the **DELAY TIME** and end of the measurement. Operation with Cell Changer: The time from the end of the **DELAY TIME** to the end of

Parameter	No.	Description
		the measurement. Within this measuring period, all samples are measured n-times consecutively. The number of cycles is determined by the measuring time and the **INTERVAL** time: Number of cycles = **TOTAL TIME/INTERVAL** + 1. If the result proves to be a decimal fraction, the next higher whole number is taken. For example: 100/30 + 1 = 4.33 = 5 cycles. The cycle time is identical to the **INTERVAL** time. The final cycle begins at the end of the measuring time. Measurement ends only on completion of the final cycle.
TRAY #	**50**	Enter the number of the tray used with the AS90/91 autosampler.
VALUE n	**8**	If the calibration curve is to be compiled from known values, (for example from the literature values or from previous measurements), these values should be entered here. Make sure that the entering sequence is the same as for the corresponding concentrations (parameter **REF**). If the calibration curve is to be recorded, do not enter anything here. After calibration, the absorbance values for the reference solutions are automatically entered. The spectrometer calculates the calibration curve from these values.
WAV.MAX	**3**	Upper limit of the wavelength range in nm. Value range: 190.0 to 1100.0; in steps of 0.1.

Parameter	N o .	Description
WAV.MIN	**4**	Lower limit of the wavelength range in nm. Range: 190.0 to 1100.0, in steps of 0.1. If peak areas are used for the calculation (see Error! Reference source not found.), the area is calculated between the upper and lower wavelength values. If calculation is made via a 2nd order derivative, the derivative spectrum is recorded between the upper and lower wavelength values.
WAVE. n	**3**	Wavelength program, Oligoquant 1: Measuring wavelength in nm. Range: 190.0 to 1100.0, in steps of 0.1. **NOTE**: The wavelengths is run in the sequence in which they are entered (when using the **ORDINATE MODE**, take **RAT** and **DIF** into account). Enter wavelengths in decreasing sequence if possible – highest first, lowest last. Concentration 1: Wavelength (n) in nm. Range: 190.0 to 1100.0, in steps of 0.1. When using **MODE – 1 WAVELENGTH**, only 1 wavelength can be entered. When using **MODE 2 WAVELENGTH**, 2 wavelengths must be entered. When using **MODE = 3 WAVELENGTH**, 3 wavelengths must be entered. **WAVELENGTH. 1** = measuring wavelength **WAVELENGTH. 2** and **3** – wavelengths for baseline correction. See **Error! Reference source not found.**

Parameter	No.	Description
WAVELENGTH	**3**	The wavelength (nm) at which measurements are made. Range: 0 (for adjustment of cell holder) 190.0 to 1100.0 in steps of 0.1.

Appendix

SuperUser

Activating SuperUser Mode

SuperUser mode gives you access to all the branches in the instrument. You activate SuperUser mode as follows:

1. Switch off the spectrometer.
2. Wait about two minutes to allow the lamps to cool down.
3. Press [1] [5] [-] (one, five, minus) simultaneously.
4. Keep the keys pressed and switch on the spectrometer.
5. Release the keys when the display appears.
   The SuperUser mode is now active.

Deactivating SuperUser Mode

1. Switch off the spectrometer.
2. Wait about two minutes to allow the lamps to cool down.
3. Switch on the spectrometer.
   The SuperUser mode is now deactivated.

Protect Functions

General Information about Protect Functions

Protect functions define the right of access to methods and branches. They can only be defined in the SuperUser mode.

You can prevent access to branches and methods using the protect function. You can set the protection so that for routine sample checking only the method dedicated to that task is accessible.

The table below lists the protect functions available in order of priority, **WRITE** has lowest priority, **ALL** (full) has highest priority.

Protect Function	Effect	Designation
Write protection	Prevents method parameters from being written over.	**WRITE**
Read/Write protection	Prevents methods from being read and from being overwritten.	**RD/WR**
Execute protection	Prevents methods from being used.	**EXECUTE**
Full protection	Prevents access to a branch and all of its methods.	**ALL**

Setting Protect Functions

Please observe the following when setting protect functions:

- Protect functions set for a method are valid for this particular method only.
- Protect functions set for a branch are automatically valid for all the methods contained in the branch.

- If a particular method has a higher protect function priority than the branch, the method protection is valid.

- If the method has a lower priority than the branch, the branch protection is valid.

For example, when a branch has read and write protection, all the methods in this branch have the same protection.

However, full protection can be set for individual methods, since full protection has a higher priority than read/write protection.

Write protection is not possible for individual methods since the branch has the higher priority.

Setting Method Protect Function

Set the method protect function as follows:

1. Switch on the spectrometer in SuperUser mode.

2. Select appropriate method.

3. Press [PARAM].

4. Use the arrow keys to select **CHANGE PROTECTION**.

5. Press [PARAM] again.

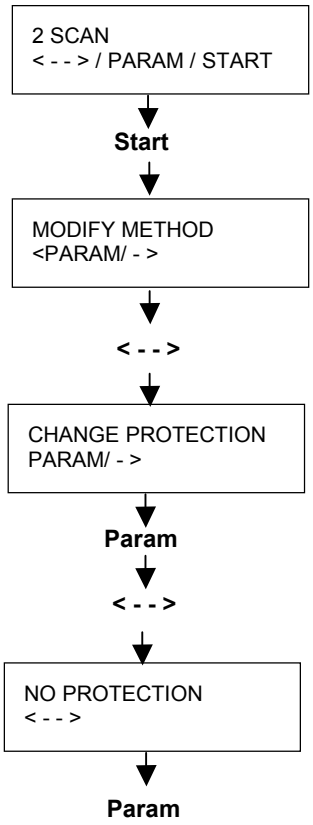
6. Use the arrow keys to select the desired protect function.

7. Press [PARAM] to confirm the protection.

   OR

   Press [STOP] to cancel.

8. Exit SuperUser mode to activate the protect function.



Setting Branch Protect Function

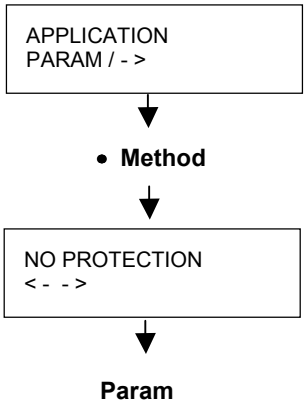
Set the branch protect function as follows:

1. Switch on the spectrometer in SuperUser mode.
2. Select the appropriate branch
3. Preset [•] [METHOD].

4. Use the arrow keys to select the required protect function.
5. Press [PARAM] to confirm the protection.

   OR

   Press [STOP] to cancel.
6. Exit SuperUser mode to activate the protect function.



Preventing Access to the Methods and Branches

You can prevent access to branches and methods using the protect functions.

Example:

If only one particular method is to be used (without access to the parameters), set the protect functions as follows:

- Set full protection for all branches except the application branch.
- Set read/write protection for the application branch.
- Set full protection for all methods in the application branch not to be used.

For the method to be used, the read/write protection of the branch is valid.

This method can be used (that is, started) outside of the SuperUser mode, but it cannot be read or altered.

Tagged parameters continue to appear when methods are being run and may then be modified.

Instrument Branches

Overview of the Instrument Branches

The basic spectrometer has 6 branches.

Branch		Content
Application	*	Analysis methods
Communication	*	Interface methods
Calibration	*	Methods to calibrate the spectrometer
Configuration	*	To set up basic spectrometer settings
Test	*	Test methods
Validation	*	Validation methods

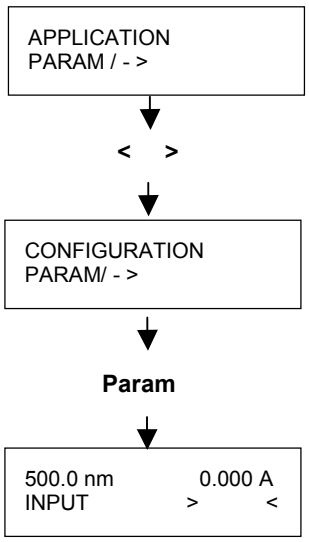
NOTE: ** can only be accessed as SuperUser.*

NOTE: *Methods can only be modified. You cannot create any new methods.*

Selecting a Branch

1. Switch on the spectrometer in SuperUser mode.
2. Press [STOP] until a branch header is displayed.
3. Use the arrow keys to select the required branch.

4. Press [PARAM].
   The branch methods can then be selected (see *Selecting a Method* on page 48).
   Can only be set in SuperUser mode.



About the Various Branches

With branches that do not contain analytical methods, you must always press [START] to make the changes effective.

APPLICATION – the Application Branch

This branch contains the analytical methods.

COMMUNICATION – the Communication Branch

This branch contains a method for controlling the spectrometer via an external computer.

No	Method	Function
29	**FREERUN**	Control of the spectrometer via an external computer. A precondition is that the computer should be equipped with a program compatible with the method in use, including UV WinLab or PECSS (version 3.1 or later). For example: To control the spectrometer using the UV WinLab software, proceed as follows: 1. Set the RS 232 interface to computer, see **RS 232 PORT CONF**. 2. Connect the computer to this interface. 3. Start UV WinLab.

CALIBRATION – the Calibration Branch

Only for service personnel (or very experienced users). This branch contains methods for wavelength calibration of the spectrometer.

No.	Method	Function
19	**0% T CALIBRATION**	Switches the dark signal compensation on and off. A residual current (dark signal) flows through the detector even when there is no beam. This signal is taken into account when the dark signal function is switched on. Compensation then takes place automatically, either at the start of a method, or every 10 minutes, whichever occurs first.
20	**ONE WAVEL. CALIB**.	Wavelength calibration with one peak. To check the calibration, record the spectrum of a wavelength standard, and compare the wavelength values recorded by the spectrometer with the values for the standard. If they do not correlate, the spectrometer should be recalibrated. Parameters: **0 nm PEAK** Internal calibration at 0 nm. **D2 PEAK** Internal calibration at 656.1 nm. **SPEC PEAK** Calibration using an external wavelength standard, for example holmium oxide.
21	**TWO WAVEL. CALIB.**	Wavelength calibration with two peaks. To check the calibration, record the spectrum of a wavelength standard and compare the wavelength values recorded by the spectrometer with the values for the standard. If they do not correlate, the spectrometer should be recalibrated.

		Parameters: **AUTO PEAK** Internal calibration at 0.0 and 656.1 nm. **SPEC PEAK** Calibration using an external wavelength standard, for example holmium oxide.

CONFIGURATION – the Configuration Branch

This branch contains methods for configuration of the spectrometer to the individual requirements of the user.

No.	Method	Function
1	**HELP CONFIG**	Level and language of help messages. Parameters: **LEVEL** Extent **LANGUAG**E Language
2	**AUTOSAMPLER CONF**	To set the autosampler parameters (including sample locations, upper and lower limits for the sampler arm). AS-90/91 autosampler parameters: **UP OFFSET** To set the up offset in mm. Range: 0 to 145 mm **DOWN OFFSET** To set the down offset in mm. Range: 0 to 145 mm **# OF ROWS** To set the number of rows for the tray. Range: 1 to 20 **X ORIG. ROW n** To set the X coordinate (left to right) of row n. Range 0 to 302

No.	Method	Function
		Z ORIG. ROW n To set the Z coordinate (back to front) of row n. Range: 0 to 185 mm **# COL. ROW n** To set the number of columns (location) in each row. Range: 1 to 20 **DISTANCE n** To set the distance from the centre of one column (location) to the next. Range: 1 to 99.9 mm **X ORIGIN FLUSH:** To set the X coordinate (left to right) of the flush solution column (location). Range: 1 to 302 nm **Z ORIGIN FLUSH** To set the z coordinate (back to front) of the flush solution column (location). Range: 1 to 185 mm For further details see the autosampler documentation.
3	**LINTRANS CONFIG**	To set the number of positions at which measurements are to be made and their location from a fixed point. Parameters: **# POSITIONS** The number of positions (1 to 20). **POSITION n** The position in mm from a fixed point (0.00 to 97.00).

No.	Method	Function
4	**COMM. CONFIG**	Protocol for the RS 232 interface for use with a PC. The default parameter values are for use with PECSS and an Epson PC. Default values: Port Enable: No ETXT-Character: 015 Respond: Prompt Prompt-Character:021 Baud Rate: 5800 Break-Character:043 Bits/Character:8 Erase-Character:010 Stopbit: 1 Kill-Character: 010 Parity: none Range: 2 Terminator: CRLF
5	**RS 232 PORT CONF.**	Usage of the RS 232 interface, marked Serial Port on the spectrometer, and the centronics interface marked Parallel Port on the spectrometer. **PRINTER PORT** Parallel Port, you can choose to have this interface active **ON** or inactive **OFF**. Select the printer using **PRINTER CONFIG.** **RS232 PORT** Serial Port, you can choose to connect a personal computer or printer to this port. **COMPUTER:** PC default setting **PRINTER:** serial printer After a full reset the value is set to **COMPUTER.**
6	**PRINTER CONFIG**	To configure the printer output. Parameters: **PRINTER ON?** Switches output to the printer on and off. **PRINTER** Selects the printer: **NO**, no printer connected **EPS, EX/FX** **EPSON LQ**

No.	Method	Function
		BIDIRECTIONAL **INTERNAL** (internal printer) **EPSON/INTERNAL** **HP-PCL** **GAP** Sets the gap between two pages. **4,6,8, FF** **PERFORATION** Skip over Perforation. Appears when **GAP** is set to **FF** (form feed). **YES, NO** **COLOR ON?** Switches color printing on and off (only for color printers). **YES, NO** **PLOT HEADER** Header printed at the start of each plot (**YES, NO**). **PAPER** When the **PRINTER** parameter is set to **EPSON/INTERNAL**, you can select Z-fold or single-sheet paper. **Z FOLD, SINGLE SHEET**

No.	Method	Function
7	**ACCESSORY CONFIG**	Accessory mode. Parameters: **ACCESSORY:** Switches accessory mode on and off. **MANUAL** To select operation with the standard cell holder. **CELL** To select the type of cell changer. **NO** is no cell changer. **CALL5, CELL6, CELL8, CELL9, CELL13** is for cell changer type. **SIPPER** To select the type of sipper. **VASI, PESI** **SI** = sipper, **VA** = vacuum, **PE** = peristaltic. **AS90/91** To select the autosampler AS-90/91 **LINTRANS** To select the linear transporter
8	**USER CONFIG**	Switches from single to double beam mode: switches background correction on and off. Parameters: **BEAM** To set the beam: **DB** Double beam **SBR** Single reference beam **SBS** Single sample beam **BASELINE CORR? YES, NO** Background correction on/off **LAMP UV** **YES, NO** Switch UV lamp on/off **LAMP Vis** **YES, NO** Switch UV lamp on/off **NOTE:** *When using single beam mode, operate only with ordinate mode %T.*

No.	Method	Function
9	**FACTORY CONFIG**	Calibration peaks offsets and filter change points. Parameters: **ONM OFFSET** Only for service personnel. Default: 0.0 nm **ABS FACT** Only for service personnel. Default: 1.0 **D2 OFFSET** Only for service personnel. Default: 0.0 nm **FILTER n** Wavelengths for filter change (filter 2-7) Default: filter 1 = 980.0 nm, filter 2 = 794.0 nm, filter 3 = 683.0 nm, filter 4 = 558.0 nm, filter 5 = 420.0 nm, filter 6 = 383.0 nm, filter 7 = 326.0 nm (lamp changeover) **LAMP** Wavelengths for lamp changeover Default: 326.0 nm **GAIN** Only for service personnel. Default: off

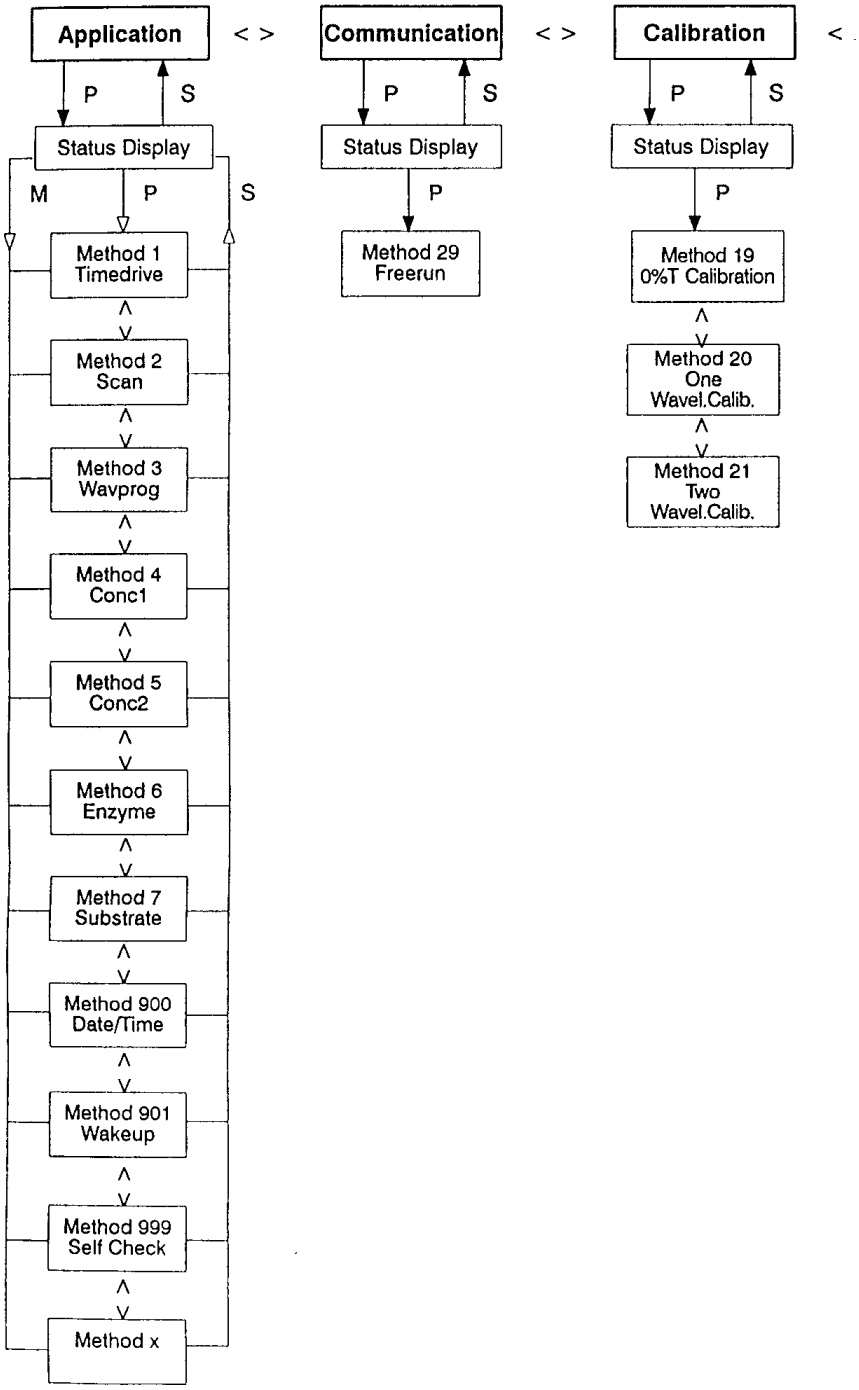
VALIDATION – the Validation Branch

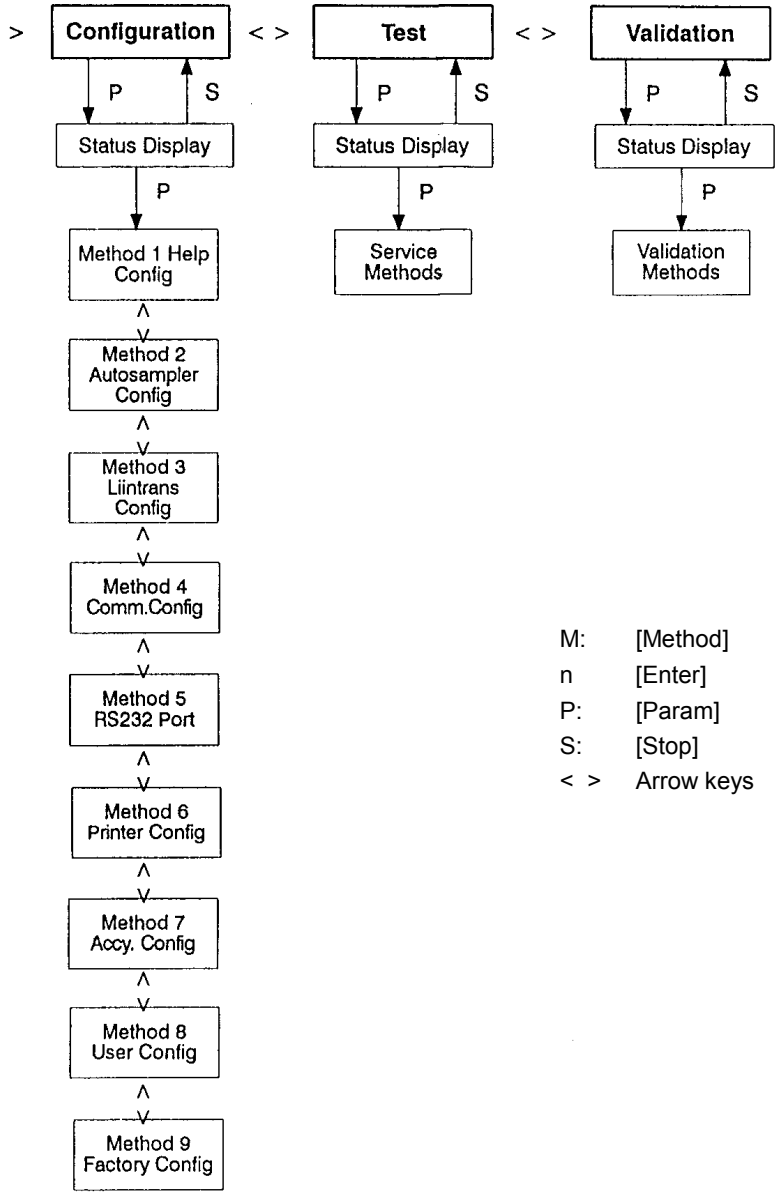
These methods are used to test that the spectrometer's performance lies within specification.

The methods in this branch are described in detail in the document *UV/Vis Spectrometer Performance Validation (*09935026) from PerkinElmer.

TEST – the Test Branch

These methods are for use by PerkinElmer Service only.


Application
< >
Communication
< >
Calibration
<
P
S
Status Display
M
P
S
Method 1
Timedrive
Method 2
Scan
Method 3
Wavprog
Method 4
Conc1
Method 5
Conc2
Method 6
Enzyme
Method 7
Substrate
Method 900
Date/Time
Method 901
Wakeup
Method 999
Self Check
Method x
Method 29
Freerun
Method 19
0%T Calibration
Method 20
One
Wavel.Calib.
Method 21
Two
Wavel.Calib.



M: [Method]
n [Enter]
P: [Param]
S: [Stop]
< > Arrow keys

Enzyme Kinetics

Enzymatic Analytical Procedures

Enzyme analysis makes use of enzyme controlled reactions to determine a substance. These procedures are used especially in clinical chemistry and food chemistry.

There are two methods of enzymatic analysis:

- The determination of enzyme concentration or enzyme activity: enzyme kinetic measurement. For example: the determination of enzymes in blood serum.
- The determination of the concentration of a substrate: substrate kinetic measurement. For example: the determination of components in food, (alcohol in wine).

The basis of both methods is the conversion of a substrate into a product whereby the enzyme acts as a catalyst.

The reaction can be followed photometrically: an added coenzyme (for example, NADH) is oxidized or reduced in the course of the reaction and the resulting change in absorbance measured. Or, the substrate or product may be photoactive and the absorbance will change with the concentration.

In enzyme kinetics, the substrate is added in excess and the reaction rate (as dA/dt) measured. With excess substrate, it is constant and directly proportional to enzyme activity.

In substrate kinetics, the substrate reacts completely. The substrate concentration can then be calculated from the change in absorbance (ΔA).

Enzyme Kinetics

In enzyme kinetics, the enzyme activity of a sample solution is determined: the sample solution containing enzyme (for example, serum) is reacted with a high excess of substrate.

The substrate is converted to product by the enzyme, the rate of the reaction can be followed photometrically and is a direct measure of the enzyme activity.

(Enzyme activity is given as International Units U:
1U = the enzyme activity required to convert 1 µmole of substrate per minute, under optimal conditions.)

The following reaction can be assumed for the conversion of substrate S into product P:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S{-}{-}E \xrightarrow{k_3} P + E$$

where:

E = enzyme and k = reaction rate constant.

The rate of reaction $S + E$ ----------> $P + E$ is governed by the Michaelis-Menten equation:

$$v = k_3 \cdot \frac{c_{0E} \cdot c_S}{k_M \cdot c_S}$$

with $v = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{dA}{dt}$ = Reaction Rate

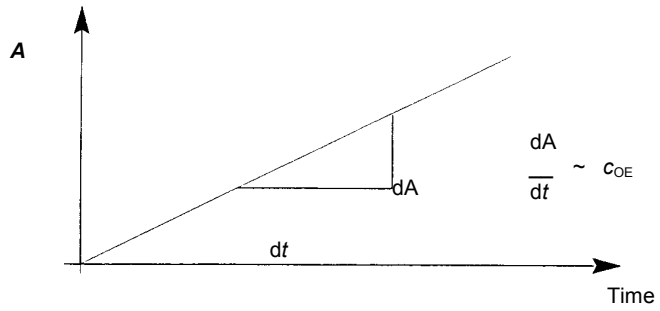
and $k_M = \frac{k_2 + k_3}{k_1}$ = Michaelis-Constant

Due to the large excess of substrate there is a limiting case:

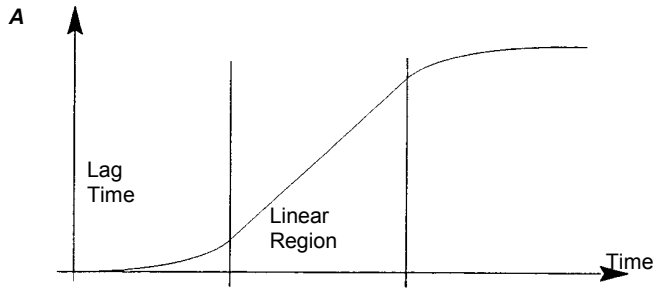
$c_S \gg k_M$ and hence

$v = k_3 \cdot c_{0E}$ is valid.

Thus, the reaction rate is constant (no consumption of enzyme) and directly proportional to enzyme concentration or enzyme activity. Hence, the enzyme activity of a solution can be directly determined by measuring the reaction rate.



In practice, the course of the curve obtained can deviate from the ideal form: it becomes linear only after a certain lag time and flattens out towards the end. In such a case, only the linear region of the curve is used for calculating the reaction rate.



Substrate Kinetics

In substrate kinetics the substrate concentration of a sample solution is determined via enzyme controlled reactions. The advantages of such a process are:

- High specificity; that is, only one substrate is converted. This avoids the necessity of complex sample preparation.
- A quicker reaction, with measuring times of only 3-30 minutes.

In the course of the reaction, the substrate is converted to product and the reaction can be followed photometrically. The reaction is started by the addition of enzyme and proceeds relatively quickly until a state of equilibrium is attained. The substrate has been converted by this time and the absorbance does not alter any more.

The measured difference in absorbance (ΔA) is directly proportional to the substrate concentration:

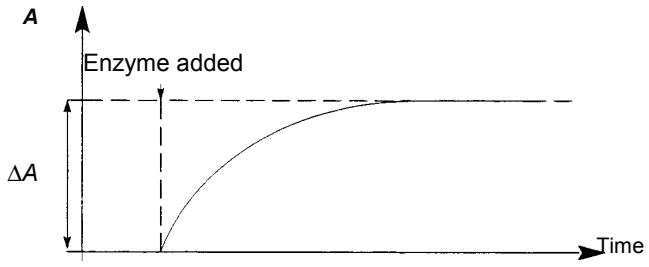
$c_{Sub} = f \,.\, \Delta \boldsymbol{A}$

where

c_{Sub} is the substrate concentration

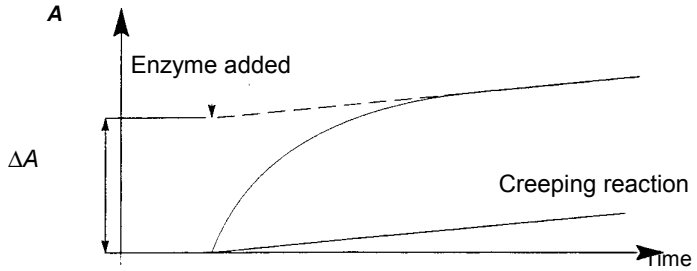
f is the concentration factor

$\Delta \boldsymbol{A}$ is the measured difference in absorbance



The course of the reaction can deviate from the ideal described above: creeping reactions can take place and the absorbance can hence increase even after the substrate reaction has been completed.

The end point of such a reaction is reached when the slope of the curve remains constant. The actual end point can then be determined by extrapolation.


A
Enzyme added
ΔA
Creeping reaction
Time

Translations of Warnings

W01.01

WARNING

Warning
We use the term WARNING to inform you about situations that could result in ***personal injury*** *to yourself or other persons.*
Details about these circumstances are in a box like this one.

D

Warning (Warnung)
Bedeutet, daß es bei Nichtbeachten der genannten Anweisung zu einer ***Verletzung*** *des Benutzers kommen kann.*

DK

Warning (Advarsel)
Betyder, at brugeren kan blive ***kvæstet****, hvis anvisningen ikke overholdes.*

E

Warning (Peligro)
Utilizamos el término ***WARNING*** *(PELIGRO) para informarle sobre situaciones que pueden provocar* ***daños personales*** *a usted o a otras personas.*
En los recuadros como éste se proporciona información sobre este tipo de circunstancias.

F

Warning (Danger)
Nous utilisons la formule ***WARNING*** *(DANGER) pour avertir des situations pouvant occasionner des* ***dommages corporels*** *à l'utilisateur ou à d'autres personnes.*
Les détails sur ces circonstances sont données dans un encadré semblable à celui-ci.

I

Warning (Pericolo)
Con il termine ***WARNING*** *(PERICOLO) vengono segnalate situazioni che potrebbero provocare* ***incidenti alle persone****. Troverete informazioni su tali circostanze in un riquadro come questo.*

NL

Warning (Waarschuwing)
Betekent dat, wanneer de genoemde aanwijzing niet in acht wordt genomen, dit kan leiden tot ***verwondingen*** *van de gebruiker.*

P

Warning (Aviso)
Significa que a não observância da instrução referida poderá causar um ***ferimento*** *ao usuário.*

C01.01

Caution	*We use the term CAUTION to inform you about situations that could result in **serious damage to the instrument** or other equipment. Details about these circumstances are in a box like this one.*
(D)	***Caution (Achtung)*** *Bedeutet, daß die genannte Anleitung genau befolgt werden muß, um einen **Geräteschaden** zu vermeiden.*
(DK)	***Caution (Bemærk)*** *Dette betyder, at den nævnte vejledning skal overholdes nøje for at undgå en **beskadigelse af apparatet**.*
(E)	***Caution (Advertencia)*** *Utilizamos el término **CAUTION** (ADVERTENCIA) para advertir sobre situaciones que pueden provocar **averías graves en este equipo** o en otros. En recuadros éste se proporciona información sobre este tipo de circunstancias.*
(F)	***Caution (Attention)*** *Nous utilisons le terme **CAUTION** (ATTENTION) pour signaler les situations susceptibles de provoquer de **graves détériorations de l'instrument** ou d'autre matériel.* *Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.*
(I)	***Caution (Attenzione)*** *Con il termine **CAUTION** (ATTENZIONE) vengono segnalate situazioni che potrebbero arrecare **gravi danni allo strumento** o ad altra apparecchiatura.* *Troverete informazioni su tali circostanze in un riquadro come questo.*
(NL)	***Caution (Opgelet)*** *Betekent dat de genoemde handleiding nauwkeurig moet worden opgevolgd, om **beschadiging van het instrument** te voorkomen.*
(P)	***Caution (Atenção)*** *Significa que a instrução referida tem de ser respeitada para evitar a **danificação do aparelho**.*

W01.02

WARNING

Unauthorized Adjustments and Servicing
Do not attempt to make adjustments, replacements or repairs to this instrument except as described in the accompanying User Documentation. Only a Perkin-Elmer service representative or similarly trained and authorized person should be permitted to service the instrument.

(D)

Unzulässige Arbeiten am Gerät
Wartungs- oder Reparaturarbeiten oder Justierungen, die in der Benutzerdokumentation zum Gerät nicht beschrieben sind, dürfen nur vom Perkin-Elmer Kundendienst oder von entsprechend ausgebildeten und autorisierten Fachkräften ausgeführt werden.

(DK)

Ikke-tilladte arbejder på apparatet
Vedligeholdelses- eller reparationsarbejder eller justeringer, som ikke er beskrevet i dokumenterne til brugeren, må kun udføres af Perkin-Elmers kundeservice eller af tilsvarende uddannede og autoriserede fagfolk.

(E)

Ajustes y servicios sin autorización
No intente realizar ningún tipo de ajuste, sustitución o reparación en este aparato, a excepción de lo descrito en la Documentación del Usuario que se adjunta. Tan sólo un ingeniero de Servicio Técnico de Perkin-Elmer o una persona de formación y autorización similares podrán realizar trabajos de revisión y mantenimiento delinstrumento.

(F)

Réglages et entretien non autorisés
Ne pas essayer d'effectuer des réglages, des remplacements ou des réparations sur cet instrument d'une manière autre que celle décrite dans la Documentation Utilisateur jointe. Seul un représentant du service après-vente Perkin-Elmer ou une personne autorisée et de même formation devraient être autorisés à intervenir sur l'instrument.

(I)

Regolazione e manutenzione non autorizzate
Non tentare di regolare, sostituire o riparare questo strumento se non come descritto nell'allegata Documentazione Utente.
Solo un rappresentante dell'assistenza Perkin-Elmer o una persona dalla analoga esperienza può eseguire, se autorizzata, la manutenzione dello strumento.

(NL)

Niet toegestane werkzaamheden aan het instrument
Onderhouds-, reparatie- of afstelwerkzaamheden die niet in de gebruikersdocumentatie van het instrument worden omschreven, mogen alleen door personeel van de klantenservice of door overeenkomstig geschoolde en geautoriseerde vakmensen worden uitgevoerd.

(P)

Trabalhos não permitidos no aparelho
Trabalhos de manutenção ou de reparação ou ajustamentos não descritos na documentação do usuário para o aparelho apenas podem ser executados pelo Serviço Técnico da Perkin-Elmer ou por pessoal especializado devidamente formado e autorizado.

W01.03

⚠ WARNING	***Explosive Atmosphere*** *This instrument is not designed for operation in an explosive atmosphere.*
(D)	***Explosionsfähige Atmosphären*** *Das Gerät darf nicht in explosionsfähigen Atmosphären betrieben werden!*
(DK)	***Eksplosive omgivelser*** *Apparatet må ikke anvendes i eksplosive omgivelser!*
(E)	***Atmósfera explosiva*** *Este aparato no ha sido diseñado para utilizarlo en atmósferas explosivas.*
(F)	***Atmosphère explosive*** *Cet instrument n'est pas conçu pour fonctionner dans une atmosphère explosive.*
(I)	***Atmosfera esplosiva*** *Questo strumento non è adatto per l'uso in atmosfera esplosiva.*
(NL)	***Explosiegevaarlijke omgevingen*** *Het instrument mag niet in een explosiegevaarlijke omgeving worden gebruikt!*
(P)	***Atmosferas explodíveis*** *O aparelho não pode ser utilizado em atmosferas explodíveis!*

W02.01

WARNING

Electrical Hazard
Any interruption of the protective conductor inside or outside the instrument or disconnection of the protective conductor (earth/ground) terminal is likely to make the instrument dangerous.
Intentional interruption is prohibited.

(D)

Gefährdung durch Elektrizität
Das Gerät muß zum Betrieb immer geerdet sein.
Auf keinen Fall die Schutzleiter im Gerät oder in der Netzzuleitung trennen oder entfernen.

(DK)

Fare på grund af elektricitet
Apparatet skal altid være jordet.
Man må under ingen omstændigheder skille eller fjerne jordlederen inde i apparatet eller i strømledningen.

(E)

Peligro eléctrico
Cualquier interrupción del conductor de protección dentro o fuera del aparato, o la desconexión del terminal del mismo (toma de tierra) podrían ocasionar serios peligros al usar el equipo.
Prohibida la interrupción intencionada.

(F)

Risque d'électrocution
Toute interruption du conducteur de protection à l'intérieur ou à l'extérieur de l'instrument, ou déconnexion du raccord du conducteur de protection (terre) peut rendre l'instrument dangereux. Il est interdit d'interrompre volontairement ce conducteur.

(I)

Pericolo: elettricità
Qualsiasi interruzione della protezione del conduttore all'interno o all'esterno dello strumento, o lo scollegamento del terminale (di terra/massa) del conduttore di protezione possono rendere pericoloso lo strumento.
È' vietato provocare volontariamente queste interruzioni.

(NL)

Risico's door elektriciteit
Het instrument moet voor de werking altijd geaard zijn. In geen geval mag de aarding van het instrument of de netvoeding worden onderbroken of worden verwijderd.

(P)

Perigo por electricidade
Para a operação o aparelho tem de estar sempre ligado à terra. De forma alguma separar ou retirar os condutores de protecção à terra no aparelho ou no cabo de alimentação da rede.

W02.02

WARNING

Lethal voltages are present in the instrument

- *Even with the power switch OFF, line power voltages can still be present within the instrument.*
- *When the instrument is connected to line power, terminals may be live, and opening covers or removing parts (except those to which access can be gained by hand) is likely to expose live parts.*
- *Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

(D)

Gefährliche Spannung im Gerät

- *Auch in ausgeschaltetem Zustand kann an einigen Stellen im Gerät Netzspannung anliegen, wenn das Gerät am Stromnetz angeschlossen ist.*
- *Auch bei ausgeschaltetem Gerät und getrennter Netzverbindung können Kondensatoren im Gerät noch mit gefährlicher Spannung geladen sein.*

(DK)

Farlig spænding i apparatet – fare for kvæstelser

- *Også i slukket tilstand kan der være netspænding nogle steder i apparatet, hvis apparatet er tilsluttet til strømnettet.*
- *Selv når apparatet er slukket, og strømforbindelsen er afbrudt, kan kondensatorerne i apparatet være ladet med farlig spænding.*

(E)

En el aparato existen voltajes letales

- *Incluso con el interruptor desconectado, puede haber voltaje dentro del equipo.*
- *Cuando el instrumento se encuentre conectado a la red eléctrica, los terminales pueden estar bajo corriente y éstos quedar expuestos al abrir las cubiertas o al extraer componentes (exceptuando aquellos a los cuales se puede acceder con la mano).*
- *Los condensadores internos del aparato pueden permanecer cargados incluso cuando el aparato haya sido desconectado del voltaje de la línea.*

(F)

Présence de tensions mortelles dans l'instrument

- *Même lorsque l'interrupteur de puissance est sur ARRET, des tensions de secteur peuvent encore être présentes dans l'instrument.*
- *Lorsque l'instrument est relié au secteur, les raccords peuvent être sous tension, et des parties sous tension peuvent être découvertes en ouvrant des capots ou en retirant des pièces (à l'exception de celles auxquelles il est possible d'accéder manuellement).*
- *Les condensateurs contenus dans l'instrument peuvent encore être chargés, même si l'instrument a été déconnecté de toutes les sources de tension.*

W02.02

WARNING

Lethal voltages are present in the instrument

- *Even with the power switch OFF, line power voltages can still be present within the instrument.*
- *When the instrument is connected to line power, terminals may be live, and opening covers or removing parts (except those to which access can be gained by hand) is likely to expose live parts.*
- *Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.*

Nello strumento sono presenti tensioni mortali

- *Anche se l'interruttore di alimentazione è in posizione OFF, la tensione di linea può essere ancora presente all'interno dello strumento.*
- *Quando lo strumento è collegato alla rete di alimentazione i terminali possono essere sotto tensione; aprendo le calotte di protezione o rimuovendo alcune parti (ad eccezione di quelle raggiungibili con la mano) è possibile esporre altre parti sotto tensione.*
- *Anche se lo strumento è stato scollegato da tutte le fonti di tensione, i condensatori al suo interno possono essere ancora carichi.*

Gevaarlijke spanningen in het instrument – gevaar van letsel

- *Ook in uitgeschakelde toestand kan, wanneer het instrument op de netvoeding is aangesloten, op sommige plaatsen in het instrument netspanning staan.*
- *Ook bij een uitgeschakeld instrument en een onderbroken netvoeding kunnen condensatoren in het instrument nog een gevaarlijke lading bevatten.*

Tensão perigosa no aparelho – perigo de ferimento

- *Mesmo desligado, o aparelho poderá ainda ter tensão de rede em alguns pontos enquanto estiver ligado à rede de corrente.*
- *Mesmo com o aparelho desligado e a ligação à rede de corrente interrompida, os condensadores dentro do aparelho ainda poderão ter ums tensão perigosa aplicada.*

W02.03

WARNING

Electrical Hazard
To prevent potential injury to yourself and damage to the instrument, switch OFF all instruments in the system and disconnect them from the line power supply before you alter, or make any new, electrical connections.

Gefährdung durch Elektrizität
Um Verletzungsgefahr durch elektrische Spannung und Beschädigungsgefahr der Geräte zu vermeiden, schalten Sie alle Geräte des Meßplatzes AUS und trennen Sie die Netzverbindungen, bevor Sie irgendwelche elektrischen Verbindungen herstellen oder ändern.

Fare på grund af elektricitet
For at undgå faren for kvæstelser fra den elektriske spænding og faren for beskadigelse af apparaterne, skal alle apparater på målepladsen være SLUKKET, og strømforbindelserne skal være skilt, inden De opretter eller ændrer noget i de elektriske forbindelser.

Peligro eléctrico
Para evitar posibles daños personales y al equipo, apague todos los módulos del sistema y desconéctelos de la toma de corriente antes de establecer nuevas conexiones eléctricas, o de modificar conexiones existentes.

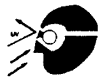
Risque d'électrocution
Afin d'éviter des blessures éventuelles et des détériorations de l'instrument, mettre tous les instruments du système hors tension et les déconnecter de l'alimentation par le secteur avant de modifier des connexions électriques ou d'en réaliser de nouvelles.

Pericolo: elettricità
Per evitare potenziali incidenti alle persone e danni allo strumento mettere in posizione OFF l'interruttore di tutti gli strumenti del sistema e scollegarli dalla fonte di alimentazione prima di modificare i collegamenti elettrici o di creare nuovi collegamenti.

Risico's door elektriciteit
Om het gevaar van letsel door elektrische spanning en het gevaar voor beschadiging van het instrument te voorkomen, schakelt u, voordat u welke elektrische verbinding dan ook wilt maken of wilt veranderen, alle instrumenten van de meetplaats UIT en ontkoppelt u de netvoeding.

Perigo por electricidade
A fim de evitar o perigo de ferimento por tensão eléctrica e o perigo de danificação dos aparelhos, DESLIGUE todos os aparelhos do posto de medição e interrompa as ligações à rede antes de estabelecer ou modificar qualquer ligação eléctrica.

W03.02



WARNING

UV Radiation–Risk of Eye Damage
The lamp emits intense UV radiation which can damage your eyes.
Do not gaze into a lighted lamp.
Always wear UV absorbing safety glasses when looking at the radiation from the lamp.

D

UV-Strahlung – Gefährdung der Augen
Die Strahlungsquelle/Lampe emittiert intensive UV-Strahlung und kann dadurch Augenschäden verursachen.
Tragen Sie eine Schutzbrille, die die emittierte UV-Strahlung ausreichend absorbiert, wenn Sie in die leuchtende Strahlungsquelle/Lampe schauen.

DK

Ultraviolet stråling – farligt for øjnene
Strålingskilden/lampen emitterer ultraviolet stråling og kan derfor forårsage øjenskader.
Bær beskyttelsesbriller, som absorberer den emitterede ultraviolette stråling tilstrækkeligt, når De ser ind i den lysende strålingskilde.

E

Radiación de rayos ultravioleta
La lámpara emite una intensa radiación de rayos ultravioleta, que puede ser perjudicial para los ojos. No mire fijamente a la lámpara.
Al examinar la radiación de las lámparas, utilice siempre gafas de seguridad que absorban este tipo de rayos.

F

Rayonnement UV
La lampe émet un rayonnement UV intense susceptible de provoquer des lésions oculaires. Ne pas regarder fixement une lampe allumée.
Toujours porter des lunettes de protection absorbant les UV pour regarder le rayonnement des lampes.

I

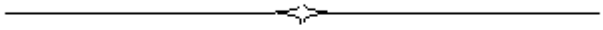
Radiazioni ultraviolette
La lampada emette intense radiazioni ultraviolette dannose per gli occhi. Non fissare le lampade accese.
Indossare sempre occhiali protettivi ad assorbimento degli ultra-violetti quando si guardano le radiazioni emesse dalla lampade.

NL

UV-straling – Risico's voor de ogen
De stralingsbron/lamp emitteert intensieve UV-straling en kan daardoor letsel aan de ogen veroorzaken.
Draag een veiligheidsbril, die de geëmitteerde UV-straling voldoende absorbeert, wanneer u in de brandende stralingsbron moet kijken.

P

Radiação UV – perigo para os olhos
A fonte de radiação/lâmpada emite uma radiação UV intensapodendo assim provocar ferimentos aos olhos.
Use óculos de protecção que absorvam suficientemente a radiação UV emitida ao olhar directamente para a fonte de radiação luminosa.



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